Broiler excreta composition and its effect on wet litter

Aspects of nutrition

Evelien van der Hoeven – Hangoor

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Thesis

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ABSTRACT

In commercial broiler farms, birds are usually housed on litter, composed of bedding materials like wood shavings. Wet litter is a condition in which the litter reaches its saturation threshold for water and cannot hold more moisture. It causes increased microbial activity and, as a result, ammonia is produced and emitted into the air. Wet litter can result in negative welfare issues (e.g., footpad dermatitis) and also a reduced performance. Wet litter is a multifactorial problem, involving management, housing, disease, diet, and gut health factors. In this thesis, nutritional aspects on excreta moisture content were studied in four different experiments. Different dietary compositions were evaluated and the results show that reductions in excreta water content were related to increased transit time and/or reduced water reabsorption in the hindgut. Insoluble fibers, if they are combined with a coarse diet, can be used to slow down transit time and optimize digestibility, thereby improving both excreta and litter quality. Minerals (e.g., Mg) and other undigested nutrients increase the osmotic load of the digesta in the hindgut and, as a result, more water is moving into the gut lumen. Changing the type or level of nutrients that reach the hindgut by varying dietary ingredients (medium-chain fatty acids, nonstarch polysaccharides, and starch) had limited effects on the ileal microbiota composition. Additionally, no effects of variations in commensal bacteria and excreta quality were observed. In this thesis, different parameters to assess the status of water in excreta and litter samples were evaluated. Water in the excreta or litter can be present in free form or bound, therefore solely total moisture content may not be sufficient to describe excreta and litter quality. Water activity correlates well with microbial growth. However, its use is limited in high moisture (> 30%) content samples. Free water, even though this parameter is dependent of the centrifugal speed applied, seems to be a more valuable parameter to assess excreta quality. The results from this thesis show that nutrition can be used to manipulate excreta and litter moisture content. The effects of nutritional manipulation can be related to (in)digestibility of nutrients, although transit time also seems to be an important factor determining excreta moisture output. Current feed strategies need to aim at optimizing the gastro-intestinal tract functions via the diet. Besides assessment of litter quality, also monitoring excreta quality throughout the growing period is highly recommended for managing litter quality, broiler health, and environmental impact. This should not be limited to measuring total excreta moisture, as the results of this thesis show that the status of the water in the excreta can be different.

CONTENTS

Chapter 1

General introduction

WET LITTER IN BROILER PRODUCTION

Broilers are usually housed on litter, primarily composed of bedding material (e.g., wood shavings) mixed with feed, feathers and excreta throughout the grow-out period (Stephenson et al., 1990; Torok et al., 2009; Dumas et al., 2011). Litter in poultry barns can vary greatly in moisture content with litter moisture concentrations ranging between 15 and 45% (Stephenson et al., 1990; Edwards and Daniel, 1992; Groot Koerkamp, 1994; Hayes et al., 2000; Miles et al., 2011b). When the amount of water added to the litter (e.g., from excreta and water spillage) exceeds the amount of water evaporated, litter moisture increases and a condition termed "wet litter" can occur. Wet litter as defined by Hermans *et al.* (2006) is "*when material covering the floors of poultry houses reaches its saturation threshold and is unable to hold more moisture*". Wet litter can result in animal welfare problems, and will be associated with production losses such as necrotic enteritis, footpad dermatitis (Harms et al., 1977; Tucker and Walker, 1992; Hermans and Morgan, 2007), and coccidiosis (Francesch and Brufau, 2004). In almost 50% of the wet litter cases, birds have been treated with antibiotics (Hermans et al., 2006). Commonly, wet litter is observed when litter moisture concentration is higher than 25 (Collett, 2012) to 35% (Ivoš et al., 1966; Eichner et al., 2007), depending on the type and amount of litter material used (Miles et al., 2011c). In practical poultry production, the incidence of wet litter in barns in the UK has been reported as high as 75% (Hermans et al., 2006).Wet litter especially occurs during the winter months when ventilation rates in the barn are lowered to save on heating costs (Tucker and Walker, 1992).

Wet litter is a multi-factorial problem, with management and housing of birds (Weaver Jr. and Meijerhof, 1991; Mitran et al., 2008), disease control (Tucker and Remus, 2001), diet (Francesch and Brufau, 2004), and gut health (Montagne et al., 2003) being the main factors involved. Figure 1 provides a schematic overview of factors that can affect bird health, digestion rate and, subsequently, excreta and litter quality. The results of a UK survey identified animal disease as the major factor (61.0%), followed by management and housing (48.4%) as causes of wet litter. Although, in 35.3% of the cases more than one factor was identified to be associated with wet litter incidence (Hermans et al., 2006). The occurrence of necrotic enteritis and wet litter problems has increased after the ban of including antimicrobial growth promoters (**AGP**) in feeds (Wierup, 2001; Tornøe, 2002; Hermans and Morgan, 2007; Teirlynck et al., 2011; Collett, 2012). It was hypothesized that these AGP controlled intestinal health of the bird to some extend (Thomke and Elwinger, 1998). Diseases such as dysbacteriosis, malabsorption syndrome, and chronic enteritis are considered to be related to

disturbed microbial composition in the proximal small intestine, resulting in poor digestion of feed ingredients (Rebel et al., 2006; Teirlynck et al., 2011). As a consequence, more nutrients are available for fermentation by bacteria capable of toxin metabolite production. Additionally, it increases excretion of nutrients in the feces and, consequently, the impact of poultry on the environment. For the poultry industry it is of great importance to be able to solve digestive and wet litter problems. Improvements in nutrient digestibility and improved gut health appear to be key drivers to reduce wet litter incidences.

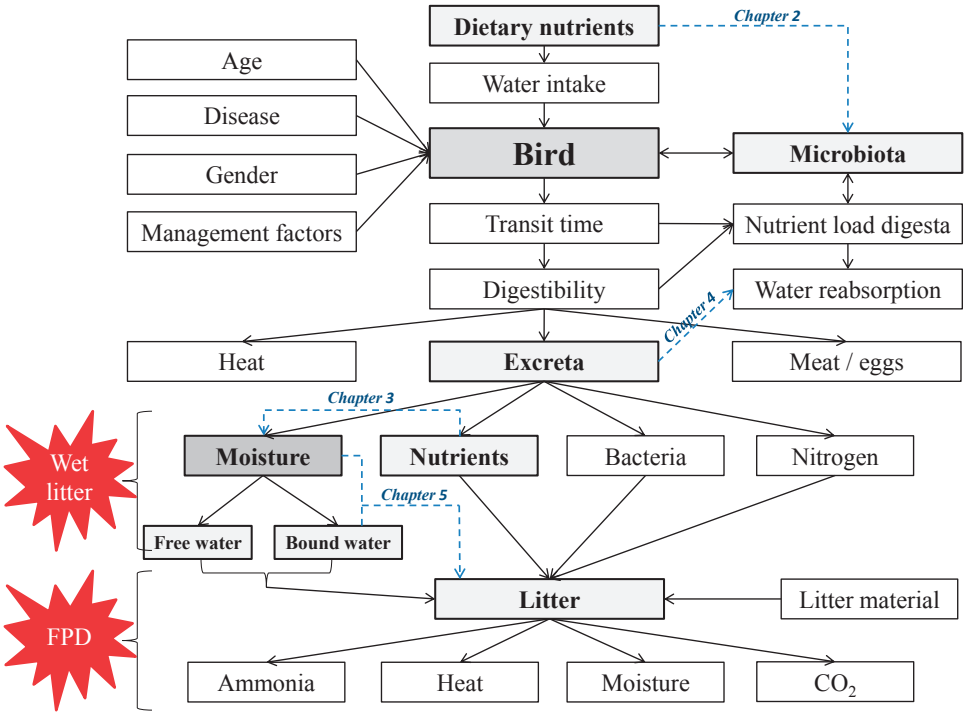

Figure 1. Schematic overview of factors affecting wet litter and footpad dermatitis (FPD). The factors studied in the different chapters are indicated.

WATER BALANCE IN THE BIRD

In birds, water intake is closely related to feed intake (Leeson et al., 1976; Lott et al., 2003). Typical water:feed ratios for broilers lay between 1.6 and 2.0 (Williams, 1996; Lott et al., 2003; Collett, 2012). Water and feed intake are regulated by the hypothalamus, in which distinct sites have been defined such as the lateral hypothalamic area and ventromedial hypothalamus. As such, injection of ß-adrenoceptors antagonists in the lateral hypothalamic region reduces water and feed intake in broilers (Baghbanzadeh et al., 2010). An overview of factors affecting water intake is shown in Table 1.

Water intake and water output are closely regulated in the body to maintain a constant hydration level in the body. The water balance changes during the growing period of a broiler. Parts of this water balance, as described by Leeson et al. (1976) and Belay and Teeter (1993), are:

Intake by *i*) water intake via drinking (65-80%), which increases with age (Alleman and Leclercq, 1997) and is highly dependent on environmental temperature and humidity (Belay and Teeter, 1996), *ii*) metabolic water production during nutrient metabolism, representing 15-25% of the water intake, and *iii*) water from the feed, generally low (<10%).

Output by *i*) water retained in the body with growth, especially high in young broilers, *ii*) water excreted via excreta, and *iii*) evaporative losses, which increase with age and is highly dependent on environmental temperature and humidity (Belay and Teeter, 1993; Ahmad and Sarwar, 2006).

Fresh droppings contain between 60-70% (Leeson et al., 1976) to 80% moisture (Henuk and Dingle, 2003). Chickens do not have a urinary bladder, and excrete urine into the cloaca (Goldstein and Skadhauge, 2000). In thermal neutral conditions of around 24°C, approximately 54% of the total water excretion occurs via the urine and 46% via the feces (Belay and Teeter, 1993). Urine production is regulated by the kidney to control the bird's hydration level. The kidneys can reabsorb 70% to 99% of the filtered water volume, resulting in less or more concentrated urine and water output (Goldstein and Skadhauge, 2000). Birds produce urine which is typically isosmotic to plasma (Goldstein and Skadhauge, 2000), although it can increase to an osmolality of 2 to 3 times higher than plasma (Laverty and Skadhauge, 2008). Also Na excretion is regulated by the kidney, where increasing dietary Na

Table 1. Factors affected water intake by broilers

Bird factors	Water quality factors	Feed factors	Housing and environmental factors
Genetics	Hardness	Feed nutrient levels	Water temperature (Xin et al., 2002)
Sex	Nitrate level	Feed ingredients (Vieira et al., 2006)	Water pressure
Age (Ross and Hurnik, 1983; Vodela et al., 1997)	Heavy metals (Vodela et al., 1997)	Feed intake (Lott et al., 2003)	Water flow rate (Lott et al., 2003; Miles et al., 2003)
Body size	Total dissolved solids	Feed form	Poorly installed regulators on drinker liner
Health status (Williams, 1996)	Bacterial contamination	Dietary water	Drinker type (May et al., 1997)
Body temperature control		Mycotoxin contamination	Drinker height (May et al., 1997)
Metabolic water production		Coccidiostats (Damron, 1994)	Spillage by broilers
			Leakage from drinker system
			House temperature (May et al., 1997)
			Air velocity (May et al., 2000)
			Air humidity

Adapted from Manning et al. (2007) and Leeson et al. (1976).

concentrations result in increased renal Na excretion (Goldstein and Skadhauge, 2000). After excretion of urine into the cloaca, retrograde peristalsis moves urine backwards into the coprodeum, colon, and ceca, where a considerable amount of water and Na from the urine can be reabsorbed (Thomas, 1982; Goldstein and Skadhauge, 2000). In a hydrated and NaCl loaded bird approximately 36% of water and 75% of Na from ureteral urine will be reabsorbed in these segments combined (Thomas, 1982). Comprehensive reviews of the avian kidney (Goldstein and Skadhauge, 2000), ceca function (Clench and Mathias, 1995; Svihus et al., 2013), and water reabsorption in the hindgut (Thomas, 1982; Goldstein and Skadhauge, 2000; Laverty and Skadhauge, 2008; McWhorter et al., 2009) are available for further detailed reading.

Disturbance of the water balance can occur by excessive water loss as with diarrhea. Diarrhea serves as a protective clearance mechanism of the intestine, triggered by inflammation or bacterial toxin invasion (Hansen and Skadhauge, 1995). In humans, net water and Na flux into the intestine were measured during acute cholera and *Escherichia coli* diarrhea, increasing digesta flow rate and water content (Banwell et al., 1972). Additionally, dietary mineral content, anion-cation balance, and several feed ingredient characteristics will affect water intake and feed passage time and as a result alter urine and fecal moisture (Collett, 2012). A thorough review of the bird's water metabolism is proved by Leeson et al. (1976) and more recently by Collett (2012).

WATER BALANCE IN THE LITTER

The main sources of water input to the litter are fresh excreta and water spillage, whereas water is removed from the litter via evaporation. Fresh excreta production by broilers is estimated at approximately 4 kg for a 35 d old broiler and 6 kg for a 49 d old broiler (Bolan et al., 2010). In laying hens housed in an aviary system, excreta production increases water content in the litter with 126.8 g water·kg^{-1} per day. At an air velocity of 1.0 m·s^{-1} and 1.0 Pa water vapor pressure difference between the air and the litter, the water content decreases daily with 94.4 g per kg litter. The result of this is a net water addition of 32.4 g·kg^{-1} in the litter per day (Groot Koerkamp et al., 1999b).

Besides moisture, poultry excreta also contain high levels of P, K, and N (Edwards and Daniel, 1992). Organic N can be transformed into aerial ammonia by bacteria and fungi

present in the litter (Carlile, 1984; Cook et al., 2011). Moisture in the litter is needed in the process of N degradation (Nahm, 2003). With increasing litter moisture concentration, more ammonia will be released, especially when the litter temperature also increases (Groot Koerkamp and Bleijenberg, 1998; Miles et al., 2011c). High ammonia concentrations can affect animal welfare through damage to the respiratory tract (Kristensen and Wathes, 2000) and through increased sensitivity to diseases like Newcastle and blindness (Carlile, 1984). As a consequence, reduced feed intake and lower feed efficiency will be observed.

Moisture content of the litter increases the occurrence of footpad dermatitis (**FPD**) (Haslam et al., 2006). There is a good relationship between litter moisture content ($R^2 = 0.62$, $P < 0.05$) and litter surface looseness ($R^2 = 0.67$, $P < 0.05$) measured at 41 d of age and hock burn occurrence in broilers (Tucker and Walker, 1992). Mayne et al. (2007) and Youssef et al. (2011b) found that litter moisture alone caused FPD in turkeys and no further aggravating effect of ammonia or uric acid was observed. Reducing excreta and, subsequently, litter moisture content as well as litter caking, therefore, is an effective measure to reduce FPD prevalence in broiler and turkey flocks (Shepherd and Fairchild, 2010).

MICROBIOTA IN THE LITTER

During a grow-out cycle of a broiler flock, the litter is constantly seeded with nutrients and microbes from the excreta, resulting in a complex litter microbiota (Cressman et al., 2010; Rothrock et al., 2010). The level of microbes in the litter can exceed 10^{10} cells per g dry litter weight (Cook et al., 2008; Rothrock et al., 2010; Dumas et al., 2011), from which approximately 77% (Dumas et al., 2011) to 90% are gram-positive bacteria such as *Actinomycetes, Clostridia/Eubacteria,* and *Bacilli/Lactobacilli* (Bolan et al., 2010). Higher litter moisture levels coincide with a higher number and diversity of microbes present as measured by DNA yield in litter samples ranging from 24% to 48% (Wadud et al., 2012) and 10 to 67% moisture (Dumas et al., 2011). Specific species (*Bacillus*, *Atopostipes,* and *Aspergillus*) were identified in these high moisture samples which could be related to odor production (Wadud et al., 2012). The microbial population in the litter regulates the release of ammonia (Carlile, 1984). Litter moisture is required for the dissolution of solid urea and subsequent urea hydrolysis (Nahm, 2003). Higher litter temperature and moisture content increase ammonia production by the microbiota and promote the volatilization of ammonia from the litter to the air (Groot Koerkamp and Bleijenberg, 1998; Liu et al., 2007b; Miles et

al., 2011c). Above approximately 55% litter moisture content, ammonia volatilization is again reduced, as the litter conditions likely become more anaerobic, limiting bacterial growth (Groot Koerkamp et al., 1998; Liu et al., 2007b; Miles et al., 2011b).

WET LITTER RISK FACTORS

Management factors

Different management factors affecting the prevalence of wet litter have been identified: ventilation (28.9%), leaking drinking systems (23.5%), adverse weather (3.7%), and leakage from the roof (2.1%) were reported as major causes of wet litter (Hermans et al., 2006). In poorly ventilated barns there is a seasonal effect on wet litter. In the winter period, the humidity in the barn is increased due to reduced ventilation rates to preserve heat loss from the house (Ivoš et al., 1966; Tucker and Walker, 1992) resulting in an increase in the litter moisture content in the barn. Other management factors that can affect litter moisture are litter type (Tucker and Walker, 1992; Miles et al., 2011c), depth of litter provided (Elwinger and Svensson, 1996), bird age (Hernandes et al., 2002; Miles et al., 2008), gender (Hernandes et al., 2002; Ziaei et al., 2007), drinker type (Elwinger and Svensson, 1996), climate (Weaver Jr. and Meijerhof, 1991), and stocking density (Tucker and Walker, 1992; Hernandes et al., 2002). Management effects on wet litter are out of scope for this thesis, although sited references and in particular Hermans et al. (2006) are available for further detailed information.

Diseases

Diseases which cause diarrhea increase the amount of water excreted by birds. In humans, bacteria can result in diarrhea via: *i*) adherence to the intestinal mucosa, followed by toxin production or invasion and infection resulting in diarrhea by peristaltic clearance movements; *ii*) toxin production, resulting in active fluid secretion by epithelial cells and increased digesta moisture content; *iii*) cytotoxin production, damaging epithelial cells by inhibition of protein synthesis and interfering with nutrient absorption; and *vi*) invasiveness by penetrating epithelial cells, resulting in bloody diarrhea with leukocytes and mucus excretion (Ashkenazi and Pickering, 1989). In poultry, non-specific enteritis (40.6%),

coccidiose (12.3%), viral infections (11.2%), dysbacteriosis (4.8%), and bacterial infections (1.1%) have been identified as major disease factors responsible for poor nutrient digestion, increased water output in the excreta and, consequently, wet litter (Hermans et al., 2006). Malabsorption syndrome, also referred to as feed-passage syndrome (Apple et al., 1991; Ruiz and De Belalcázar, 2005), has been described as an infectious (combination of virus and bacteria) cause of wet litter, resulting in impaired feed efficiency and wet and slippery litter with visible undigested ingredient particles (Kouwenhoven et al., 1992; Songserm et al., 2000; Rebel et al., 2006). Challenging birds with coccidiosis lowered water retention compared with healthy control birds (100 to 87%). In addition, more gut lesions and a subsequent reduced gut integrity were observed in coccidiose-infected birds (Tucker and Remus, 2001).

Gut health

Three major factors are important for gut health: diet, the mucosa, and the commensal flora (Montagne et al., 2003). The commensal flora, or microbiota, is one of the largest metabolically active "tissues" of the chicken (Apajalahti, 2005). Bacterial density and diversity increases from the proximal to the distal gastrointestinal tract (**GIT**) segments, as shown in Table 2. A comprehensive review by Rehman et al. (2007) on bacteria throughout the broiler GIT is recommended for further reading.

Guarner and Malagelada (2003) reviewed three main functions of the microbiota: *i*) metabolic, including short chain fatty acid (**SCFA**) production from fermentation of dietary nutrients from undigested compounds in the small intestine or from nutrients that were not directly absorbed by the host after digestion due to a too slow absorption rate, *ii*) trophic, by means of interacting with and training of the host's immune system, and *iii*) protective, through competing with exogenous or pathogenic bacteria for attachment sites on the gut wall or nutrients for growth. The trophic function of the microbiota is outside the scope of this thesis, although excellent reviews on the avian gut microbiota (Kohl, 2012) and the interaction between microbes and the immune system (Brisbin et al., 2008) are available and recommended for more detailed reading.

The microbiota use dietary nutrients for growth and reproduction, competing with the host for valuable nutrients (Apajalahti et al., 2004). It has been estimated that the small

intestinal flora can use 10 to 20% of the nutrients that would otherwise be available to the host (Apajalahti et al., 2004). In the chicken hindgut, the 2 main groups of bacteria can be classified as protein (putrefactive bacteria) and carbohydrate (saccharolytic bacteria) fermenting types. Fermentation of protein produces metabolites that are, in general, harmful for the host and increase digesta pH. Carbohydrate fermentation yields mostly SCFA that reduce intestinal pH and promote gut health. Therefore, carbohydrate fermenting bacteria are considered beneficial microbes (Apajalahti, 2005). Dietary changes will affect the substrate at the distal GIT and the diversity of bacterial species of the microbiota (Bedford, 2000; Rehman et al., 2008).

Table 2. Bacterial density in colony forming units (CFU) per gram in different gastrointestinal tract

Item	Bacterial density		Bird age, d	Reference
	log CFU	CFU·g^{-1}		
GIT segment				
Crop	5.6	3.8×10^5	unknown	Smith and Berrang (2006)
Gizzard	2.9	7.5×10^2	unknown	Smith and Berrang (2006)
Duodenal loop	6.0	1.11×10^6	47	Dumonceaux et al. (2006)
Mid-jejunum	5.4	2.75×10^5	47	Dumonceaux et al. (2006)
Ileum	7.2	1.63×10^7	47	Dumonceaux et al. (2006)
		10^9	35	Apajalahti et al. (2004)
		10^8-10^9	30	Gong et al. (2002)
Ileo-cecal junction	7.4	2.55×10^7	47	Dumonceaux et al. (2006)
Ceca	8.4	2.75×10^8	47	Dumonceaux et al. (2006)
		10^{11}	35	Apajalahti et al. (2004)

Diets differing in type and level of nonstarch polysaccharides (**NSP**; Santos et al., 2007) or including prebiotics (Rehman et al., 2009; Yang et al., 2009) have been proven successful in changing both growth performance and microbiota composition in chickens. The effect of dietary fibers on the microbiota depends on their solubility and on the rate of their digestion. Soluble fiber fractions can change the microbiota notably (Langhout et al., 2000; Hetland et al., 2004). Insoluble fibers will only be partly degraded by bacterial fermentation and have only a minor effect on microbiota composition (Choct et al., 1996). Furthermore, rapidly fermentable carbohydrates are digested early in the GIT, whereas slowly fermentable carbohydrates may affect the microbiota residing in the lower gut (Rehman et al., 2009). The use of exogenous dietary NSP degrading enzymes (e.g., xylanase, glucanase) increases the availability of dietary compounds for further digestion. Soluble NSP increase viscosity of the

digesta, thereby reducing digestibility of starch, fat, and protein (Bedford and Morgan, 1996). The enzymes break these NSP fractions into smaller polymers, thereby reducing their viscous properties and improving nutrient digestibility (Owens et al., 2008; Yang et al., 2009).

An animal has several mechanism to control bacteria levels in the intestine; *i*) wash out of luminal bacteria by increasing passage rate, or by *ii*) secretory diarrhea, *iii*) mucosal or epithelial renewal to remove adherent bacteria (Apajalahti, 2005), and *iv*) competition for nutrients, by quickly absorbing nutrients. In pigs, secretory diarrhea reduced several bacterial populations and resulted in large changes in relative presence of bacterial groups in the lumen of the ileum, ceca, and colon (Oli et al., 1998). Limited information on the effect of beneficial microbes on excreta quality is available. Although, no effect of feeding a probiotic (*Bifidobacterium longum*) on fecal water contents was observed in humans (Benno and Mitsuoka, 1992).

Dietary factors

Diet can be either beneficial or harmful to the animal with regard to gut health (Montagne et al., 2003). Dietary composition and chemical properties as well as animal related factors determine the digestibility of nutrients and, subsequently, the amount of surplus nutrients that are excreted in the feces. Among the dietary composition characteristics, many factors play a role in excreta composition, e.g., mineral levels and mineral ratios, protein content, and fiber fractions. Furthermore, dietary properties can have an impact on nutrient digestibility, diet passage time through the GIT, and excreta moisture content.

Dietary electrolyte balance

Practical poultry diets are formulated with the aim to have a certain dietary electrolyte balance (**dEB**). Essential in the dEB calculation are Na^+, K^+, and Cl^-. The dEB expresses the ability of Na^+ and K^+ to neutralize hydroxyl groups (OH^-) and of Cl^- to neutralize hydrogen ions (H^+). In the calculation, the atomic weight of the elements is taken into account and, therefore, the dEB level is expressed in milliequivalent (mEq). One limitation of using dEB is that the source of the ions is not considered. Therefore, dEB cannot solely be used to evaluate effects of different mineral sources (Ahmad and Sarwar, 2006). Increasing dEB increases excreta moisture levels (Ahmad et al., 2009), likely due to increased Na (Smith et al., 2000a;

Ravindran et al., 2008) or K intake (Ahmad and Sarwar, 2006). Increasing dietary K levels resulted in an increase in excreta moisture, whereas no effect of dietary Cl on excreta moisture was found (Koreleski et al., 2010; 2011). The dietary salt content affects the blood osmotic pressure, which is a thirst regulating mechanism (Borges et al., 2003a). As a result, broilers will consume more water and, consequently, this will result in extra water output via the excreta (Vena et al., 1990; Smith et al., 2000b). There can also be an active Na excretion via the kidneys (natriuresis). An excess of dietary mineral levels significantly increases the osmolality of the blood, resulting in a decreased water re-absorption by the kidneys (Vena et al., 1990). An increase of dietary Ca or P levels did not show consistent effects on excreta moisture (Ferguson et al., 1998b; Smith et al., 2000b; Ziaei et al., 2008). The latter may be related to the low electrolytic capacity of divalent ions compared with monovalent ions. In this, the electrolytic capacity of trace minerals is the lowest (Ahmad and Sarwar, 2006). Dietary addition of the trace minerals Mn (5 to 100 $mg \cdot kg^{-1}$), Zn (20 to 225 $mg \cdot kg^{-1}$), and Cu (3 to 19 $mg \cdot kg^{-1}$) did not clearly affect excreta moisture level (Zhong et al., 2007).

Protein

It has been shown that too high protein content (so-called over-formulation of diets) or an imbalanced amino acid profile can cause wet litter (Collett, 2006; Namroud et al., 2009). The excess protein is catabolized and used as energy source with the N atom in the amino acids excreted as uric acid by birds (Namroud et al., 2008). Uric acid is highly insoluble (Scott et al., 1976) and is present in a colloid suspension which does not contribute to water excretion (Goldstein and Skadhauge, 2000; Namroud et al., 2009). High dietary protein is, however, associated with an increased water consumption, which can result in extra water excretion in the feces (Elwinger and Svensson, 1996; Alleman and Leclercq, 1997). It has been suggested that the effect of protein on excreta moisture is confounded with the source of protein in the diet (Tucker and Walker, 1992; Francesch and Brufau, 2004). In Europe, protein in broiler diets is usually supplied using soybean meal (**SBM;** Nagaraj et al., 2007). Soybean meal, a vegetable protein source, is known to have a high K content (Alleman and Leclercq, 1997). In addition, SBM also contains certain antinutritional factors including indigestible oligosaccharides and pectins (Choct et al., 2010; Faber et al., 2012), saponins, and trypsin inhibitors (Frikha et al., 2012). These SBM soluble oligosaccharides increase water intake (Elwinger and Svensson, 1996; Vieira et al., 2006) and, subsequently, excreta (Vieira

and Lima, 2005; Vieira et al., 2006) and litter moisture. Also poorer visual score of the excreta has been observed (Leske et al., 1991; Youssef et al., 2011a). Furthermore, wet droppings, nonspecific enteritis, and feed passage were observed in commercial broiler breeder flocks when SBM trypsin inhibitor content is above 3 $g \cdot kg^{-1}$ (Ruiz and De Belalcázar, 2005; Ruiz, 2008).

Carbohydrates

In poultry diets, carbohydrates in the form of starch are the main energy source. Dietary fibers (**DF**) are in general recognized as 'anti-nutritive' because they impair digestibility of various nutrients (Montagne et al., 2003; Hetland et al., 2004). However, there are also DF types that act as a prebiotic and are able to indirectly promote gut health. Rapidly fermented DF provide substrates for the synthesis of SCFA (Jamroz et al., 2002; Józefiak et al., 2004). Slow or incomplete fermentation of DF reduces transit time through the GIT, increase fecal weight and improve laxation (Dikeman and Fahey Jr, 2006). Dietary fibers consist of NSP and non-carbohydrate compounds (Józefiak et al., 2004). Nonstarch polysaccharides can be classified into soluble (gums, pectins, psyllium, and ß-glucans) and insoluble (lignin and cellulose) fiber fractions (Dikeman and Fahey Jr, 2006). In general, soluble NSP are easily and nearly completely fermented when compared with insoluble NSP.

Soluble NSP are viscous dietary fibers (Jamroz et al., 2002) and have anti-nutritive activity in broiler chickens. Viscous fibers reduce ileal and fecal digestibility, either by limiting the access of digestive enzymes to important feed nutrients and hydrolyze them into smaller absorbable molecules and/or by thickening the GIT contents by forming gel matrixes and increasing GIT transit time (Wang et al., 1992; Montagne et al., 2003; Dikeman and Fahey Jr, 2006). Viscous fibers can result in softer feces and may lead to problems concerning wet litter (Ouhida et al., 2000). In addition, the viscosity of the digesta increases further along the GIT. As a consequence, NSP fermentation occurs mostly in the distal part of the GIT and lowers pH values (Hetland et al., 2004). A low pH will inhibit certain pathogenic bacteria and promotes gut health.

Insoluble fibers reduce transit time through the GIT, improve water-holding capacity, and assist in bulking of the feces (Jørgensen et al., 1996; Montagne et al., 2003). Furthermore, gizzard size and activity clearly increases after inclusion of insoluble fiber fractions in diets,

resulting in improved total tract starch digestibility and decreased gizzard pH (Hetland et al., 2002; Hetland et al., 2003; Amerah et al., 2009; Jiménez-Moreno et al., 2013a). Increased amylase and bile salt concentration in the gizzard were observed, suggesting a higher duodenal reflux to the gizzard (Hetland et al., 2003). The bile acids act as emulsifiers, facilitating the solubility of lipids released by protein degradation (Hetland et al., 2003; Hetland et al., 2004; Choct, 2009). The decreased gizzard pH additionally prevents potential pathogenic bacteria from entering the intestinal tract (Engberg et al., 2004; Józefiak et al., 2004).

Most of the experiments described evaluated the effect of a single nutrient on digestibility and excreta moisture. Observations including multiple nutrients are scarce. Tucker and Walker (1992) tested dietary Na content (0.13 and 0.27%) in combination with increased lysine content (1.10, 1.26, and 1.46%) and protein quality (poor, being poultry by-product meal and meat and bone meal, and good, being de-hulled and full fat soya and herring meal). Litter moisture increased in the low Na diet with increasing Lys content (from 42.0 to 47.2%) and feeding a poor protein quality (42.5 to 48.1% for good and poor protein quality, respectively). Feeding a 0.27% dietary Na increased litter moisture compared with feeding 0.13% dietary Na, however, no additional effect of protein quality or Lys content were found. These results indicate that the effect of Na on litter moisture content is greater than the effect of protein level or quality. However, the effects of the three factors seemed additive for the percentage hock burns.

AIMS AND OUTLINE

Because wet litter is a multi-factorial problem, and interactions between dietary nutrients and excreta quality are not fully understood at present, it is important to combine all available knowledge on individual feed compounds and conduct a risk-assessment for farmers to be able to estimate chances of broilers to develop wet litter problems based on excreta nutrient levels. The aim of this thesis was to study excreta moisture output via diet, thereby providing nutritional means to reduce wet litter incidences and improve broiler welfare. Furthermore, different measurements to assess excreta quality are studied. Management and disease risk factors are outside of the scope of this thesis.

Hypotheses:

1. Changing dietary composition affects digestibility and excreta moisture content in broilers.

2. The interactions between dietary nutrients and chemical compounds are more important than single nutrients for excreta moisture content in broilers.

3. Minerals can have different effects on excreta moisture level when fed as different sources (e.g., oxide vs. sulfate) or at different inclusion levels to broilers.

4. Excreta moisture level alone is not sufficient to describe excreta quality.

In **Chapter 2** the effect of diet on microbiota composition and excreta moisture level were studied. Diets differing in raw material composition (corn vs. wheat, enzyme vs. no enzyme, regular starch level vs. resistant starch), which previously have been related to excreta moisture content were fed to broilers and the effect of these diets on GIT microbiota composition and excreta quality of broilers were measured.

Chapter 3 models the relationships between excreta nutrient (nitrogen, NDF, and minerals) content and excreta moisture content to determine nutrients or combinations of nutrients potentially promoting high excreta moisture level. The aim of this statistical exercise was to find causal relationships between excreted nutrients and excreta moisture that can be used to explain variation in excreta moisture and to adapt dietary compositions.

In **Chapter 4** the effects of different dietary Mg salts (chloride, oxide, and sulfate) and inclusion levels on digesta and excreta moisture content in broilers were measured. Furthermore, the possibility to measure excreta free water level as an alternative measure to evaluate excreta quality and how it varies according to the different dietary treatments was studied.

In **Chapter 5** the possibility to assess excreta and litter quality using free water content and water activity measurements were studied by feeding different dietary compositions that were likely to affect intestinal water reabsorption and moisture excretion.

Chapter 2

Ileal microbiota composition of broilers fed various commercial diet compositions

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ABSTRACT

Microbiota plays a role in the release and absorption of nutrients from feed components, thereby affecting digesta composition and moisture content of the excreta. The objective of the current study was to determine the effects of 5 different diets varying in ingredients (medium-chain fatty acids, nonstarch polysaccharides, and starch) on the microbiota composition of ileal digesta of broiler chickens and excreta DM content. Each treatment was repeated 6 times in cages each containing 18 Ross 308 broilers, with growth performance measured from 0 to 34 d of age and excreta DM and ileal microbiota composition analyzed at 34 d of age. Microbiota composition was evaluated using a novel ribosomal RNA microarray technology containing 370 different probes covering various genera, groups of microbial species, and individual species of the chicken gut microbiota, of which 321 had a signal above the background threshold. Replacing part of the animal fat and soybean oil in the wheat-based diet with medium-chain fatty acids (MCFA; 0.3% C10 and 2.7% C12) improved feed efficiency compared with the other dietary treatments. This coincided with a suppression of gram-positive bacteria belonging to the phylum of the *Firmicutes,* including *Lactobacillus* species, and species belonging to the family of the *Enterococcaceae* and *Micrococcaceae,* while the gram-negative bacteria belonging to the family of the *Enterobacteriaceae* were promoted. None of the other diets used in the present study notably changed the ileal digesta bacteria composition. Excreta DM content was not affected by dietary treatment. The variation between individual birds per dietary treatment was more pronounced than variation caused by feed composition, with the exception of the digesta microbiota of the birds fed the MCFA diet. It is concluded that a diet with MCFA significantly changes the ileal microbiota composition, whereas the effect of the other diets on the composition of the microbiota and excreta DM content is small in broiler chickens.

Keywords: broiler, feed composition, microbiota, microarray, excreta dry matter

INTRODUCTION

Microbiota plays an important role in broilers from the moment of hatch onward. Intestinal bacteria are important for priming and maintaining an active immune system (Kelly and King, 2001; Kohl, 2012), modulation of gut function, and protection against pathogen colonization (Kelly and King, 2001). After hatch, the microbiota of a bird develop rapidly and a stable flora is achieved at approximately 2 wk of age (Rehman et al., 2007). However, large variation in microbiota composition between individuals within the same management conditions and fed the same diet has been observed (Apajalahti et al., 2001; Owens et al., 2008; Torok et al., 2011). The microbiota composition can be affected by environmental factors, genetics, and substrate availability within in the gut (Vahjen et al., 1998; Apajalahti et al., 2004). Substrate can be of dietary or endogenous origin, such as sloughed-off epithelial cells, enzymes, or mucus (Kelly and King, 2001). Studies have shown that the microbial composition in the ileum of broilers affect intestinal function, digestion, and nutrient absorption (Gong et al., 2002; Hübener et al., 2002). Ileal digesta of broilers contains between 10^8 and 10^9 bacteria per gram (Gong et al., 2002; Apajalahti et al., 2004) with *Lactobacillus* spp. (70%), *Clostridiaceae* (11%), *Streptococcus* spp. (6.5%), and *Enterococcus* spp. (6.5%) being most abundant (Lu et al., 2003). Previous analysis by 454 16S rDNA amplicon sequencing in another experiment from our group showed the dominant presence of *Lactobacillus* spp. in the ileum. Additionally, present in the ileum, although at lower ratios, are *Streptococcaceae, Enterococcaceae, Staphylococcaceae, Enterobacteriaceae, Clostridiaceae, Coriobacteriaceae, Peptostreptococcaceae,* and *Micrococcaceae*.

Unabsorbed dietary nutrients at the end of the small intestine are a potential substrate for the microbiota in the distal gastrointestinal tract (**GIT**; distal ileum and ceca; Apajalahti et al., 2004). Analysis of cecal microbial composition of birds from different commercial farms showed that feed composition is an important source of variation to modulate microbiota composition (Apajalahti et al., 2001). Dietary changes affect substrate availability and also the composition of bacterial species within the microbiota at the distal GIT (Bedford, 2000; Rehman et al., 2008). Diets differing in type and level of nonstarch polysaccharides (**NSP;** Santos et al., 2007) or containing prebiotics (Rehman et al., 2009; Yang et al., 2009) have been shown to be successful in changing both GIT microbiota composition and growth performance of broilers. Feeding wheat compared with corn-based diets increased small intestinal microbial diversity in broilers (Hübener et al., 2002) and turkeys (Santos et al., 2008). Rapidly fermentable carbohydrates are hydrolyzed by the microbiota in the proximal

GIT, whereas slow fermentable carbohydrates are hydrolyzed by the gut microbiota present in the distal GIT (Rehman et al., 2009), thereby exerting their effects in different GIT segments. The use of exogenous dietary NSP degrading enzymes can alter digesta viscosity in chickens (Owens et al., 2008; Yang et al., 2009), DM content of ileal digesta (Bergh et al., 1999), and influence substrate availability for the microbiota resulting in changes in the microbiota in the distal GIT (Bedford, 2000).

The majority of experiments reported in the literature investigating bacterial populations in the GIT of broiler chickens were based on culturing or molecular fingerprinting techniques (Gong et al., 2002; Hübener et al., 2002; Knarreborg et al., 2002). The disadvantage of these techniques is the underestimation of microbial abundance and diversity (Kohl, 2012). It was estimated that 90% of bacterial species cannot be cultured in laboratory conditions (Apajalahti et al., 2004). Furthermore, fingerprint techniques fail to identify the taxonomic identity of specific bacteria (Apajalahti et al., 2001; Gong et al., 2002; Hill et al., 2002; Torok et al., 2011). The extent to which the microbiota composition changes due to commonly used feed compositions and additives is, therefore, largely unknown (Kohl, 2012). There have been major advances in technologies for microbiota characterization in the past years, which can better overcome the above-mentioned limitations.

The present study aimed at characterizing the effect of different diets varying in ingredients (medium-chain fatty acids, nonstarch polysaccharides, and starch) on the microbiota composition of ileal digesta as well as excreta moisture content of broiler chickens at 34 d of age. In addition, the impact of the dietary treatments on production performance traits was evaluated, which is an important measure for practical broiler production. A novel ribosomal RNA microarray technology containing probes specific for the various bacterial species and groups of species present in the GIT of broilers was used to characterize microbiota.

MATERIALS AND METHODS

Birds and Housing

The experiment was performed in a broiler unit consisting of one room with 30 cages, divided over 6 blocks of 5 cages each. Five hundred forty Ross 308 male 1-d-old chicks, derived from 52-wk-old broiler breeders, were purchased from a commercial hatchery (Lunteren, the Netherlands) and allocated across the 30 cages in such a way that each treatment was represented in each block. Each cage consisted of 18 birds and had similar total weights per cage within block. Average initial individual chick weight was 39.6 ± 0.15 g.

The cages (100 x 110 cm) had a raised wire floor on top of which a rubber plate was placed, which was covered with a 2-cm layer of wood shavings. Each cage was equipped with 2 adjustable cup drinkers and a feeder that was positioned inside the cage for the first 13 d. From d 14 onward, feed was supplied via a feeder trough in front of the cage. Both feed and water were provided ad libitum throughout the study. Continuous artificial lighting was maintained for 23 h/d throughout the experiment. Temperature, relative humidity, and ventilation were computer controlled with the temperature gradually decreasing by 2.5°C per week, from 34°C on the day of arrival (1-d-old chicks) to a final temperature of 20.5°C at the end of the experiment (d 34). Room temperature was recorded continuously using data loggers, and relative humidity was set at 50% throughout the experiment. The birds were spray-vaccinated against Newcastle disease (Poulvac NDW-vaccine) at 13 d of age. The experimental methods were approved by the Ethical Committee of the Animal Science Group of Wageningen University and Research Center, Lelystad, the Netherlands.

Experimental Diets

The 5 diets per growing phase were formulated to meet the nutrient requirements of broilers (CVB, 2006) and to contain similar contents of ME and apparent fecal digestible lysine, methionine + cystine, threonine, and valine. The starter and grower diets contained 2,850 and 2,900 kcal of AME·kg^{-1} and 10.48 and 9.87 g·kg^{-1} apparent fecal digestible lysine, respectively. In advance of diet formulation, batches of wheat, corn, soybean meal, soybean isolate, fish meal, rye, toasted soybeans, and rapeseed meal were reserved and analyzed for CP (Combustion, ISO 16634, Rapid N Cube, Elementar GmbH, Hanau, Germany). Near-infrared reflectance spectroscopy analysis (Bruker MPA, ISO 12099, Bruker Optik GmbH,

Ettlingen, Germany) was applied to estimate DM, crude fat, crude fiber, and crude ash content. In addition, soy protein concentrate, rapeseed meal, and rye were analyzed for Ca, P, Na, K, Mg, Zn, Cu, Mn, Fe content (ICP-AES, ISO 27085:2009, Thermo Iris Intrepid II XSP Duo, Thermo Scientific Inc., Waltham, MA), and Cl content (Ion chromatography Dionex DX-120, Thermo Scientific Inc.; Smee et al., 1978). Finally, both the soy protein concentrate and the soybean meal were analyzed for their amino acid content (ISO/IEC 17025; TNO, Zeist, the Netherlands).

The first experimental diet (**CO**) was based on 65% corn, which was considered a digestible diet for a broiler with a low NSP content. For the second experimental diet (**ST**), the amount of slow digestible starch was increased by adding 15% pure corn starch containing 70% amylose (Hylon VII, National Starch, Manchester, UK) to the CO diet at the expense of corn and subsequently fine-tuning the diet with wheat, SBM, animal fat, soya oil, mineral sources, and synthetic amino acids to ensure an isonutritious and isocaloric diet. It was expected that this would increase the amount of undigested starch at the ileal level and thus also change the bacterial flora. The third experimental diet (**WHE**) was formulated using wheat (41.5%), rye (6.5%), and rapeseed meal (6.5%) to obtain a high level of NSP. For the fourth experimental diet (**ENZ**), an enzyme complex (Hooge et al., 2010) was added (200 $mg \cdot kg^{-1}$; Allzyme SSF, Alltech Inc., Nicolasville, KY) to the WHE diet. It was expected that this enzyme addition changes digesta viscosity and microbial composition compared with the WHE diet. For the final experimental diet (**MCFA**), 0.3% C10 (capric acid) and 2.7% C12 (lauric acid; Chempri, Raamsdonkveer, the Netherlands) were added to the WHE diet at the expense of soybean oil and animal fat. Previous experiments (E. van der Hoeven-Hangoor, unpublished) showed beneficial effects of MCFA on intestinal health and feed conversion ratio (**FCR**). The ingredient and chemical composition of the experimental diets is provided in Table 1. Diets were pelleted with steam addition (approximately 80°C) at 2.5 mm (starter diets) and at 3.0 mm (grower diets). After production, all diets were analyzed for CP (combustion, ISO 16634), crude fat (AOCS Am 5-04, Ankom XT15, Ankom Technology), crude fiber (AOCS Ba 6a-05, Ankom A 200, Ankom Technology, Macedon, NY), DM (Gavimetry, ISO 6496, Memmert UNB 500, Memmert GmbH, Schwabach, Germany), and Ca and P (ICP-AES, ISO 27085:2009. Thermo Iris Intrepid II XSP Duo, Thermo Scientific Inc.) content.

Data Collection

Bird weights were recorded per pen at the start of the experiment (d 0) and for individual birds at 6, 13, 20, 27, and 34 d of age. In addition, feed consumption for each pen between weighing was recorded on the same days as the birds were weighed. Based on BW gain and feed consumption, FCR (kg of feed consumed/kg of weight gain) was calculated. Additionally, a corrected FCR was calculated for a final body weight of 1,804 g using a correction factor of 0.03 points for each 100 g weight difference (WUR, 2011).

At 34 d of age, the litter and the rubber plate were removed from all cages, leaving the birds on the wired floor, and metal plates were placed underneath the cages to facilitate excreta collection. All excreta were collected per cage with feathers and feed particles carefully removed. Excreta samples were homogenized directly after collection, pH was measured by inserting a pH-electrode (CyberScan pH 11, Eutech Instruments Pte Ltd., Singapore) immediately after collection, and a representative subsample was taken for DM analysis (predried at 70°C for 16 h, ground, and subsequently dried at 103°C for 4 h). Additionally, 3 broilers were randomly selected from each cage and weighed before being killed by cervical dislocation. From these birds, the pH of the digesta in the mid-ileum (halfway between Meckel's diverticulum and ileal-cecal junction) and of the ceca were determined by inserting a pH electrode (CyberScan pH 11, Eutech Instruments Pte Ltd.) immediately after exposure of the GIT segment. Data of the 3 birds per cage were averaged. Furthermore, digesta was collected from the mid-ileum. For profiling the microbiota in the mid-ileum using a polygenetic microarray targeting 16S rRNA gene sequences, 16 individual birds per treatment were randomly selected based on available total space on the microarray (80 in total).

Table 1. Ingredient and nutritional composition of the experimental diets

	Starter diet (0-13 d)					Grower diet (14-34 d)				
Item	Corn	Starch	Wheat	Enzyme	MCFA[1]	Corn	Starch	Wheat	Enzyme	MCFA[1]
Ingredient composition, g·kg^{-1}										
Corn	650.4	452.4	100.0	100.0	100.0	650.0	475.0	100.0	100.0	100.0
Wheat	50.0	75.0	415.2	415.0	419.6	50.0	8.9	457.9	457.7	464.4
Rye	-	-	65.0	65.0	65.0	-	-	65.0	65.0	65.0
Rapeseed meal (XP<380)	-	-	65.0	65.0	65.0	-	-	65.0	65.0	65.0
Soybean meal HP	199.8	224.4	122.4	122.4	121.6	206.3	294.0	118.9	118.9	117.6
Soya isolate	30.0	30.0	40.0	40.0	40.0	33.8	2.3	45.0	45.0	45.0
Fish meal	20.0	20.0	20.0	20.0	20.0	-	-	-	-	-
Toasted soybeans	-	-	50.0	50.0	50.0	-	-	50.0	50.0	50.0
Animal fat	1.6	1.0	39.2	39.2	22.4	12.9	20.0	44.1	44.1	17.7
Soybean oil	1.6	1.0	39.2	39.2	22.4	4.3	6.7	14.7	14.7	5.9
Premix starter[2]	10.0	10.0	10.0	10.0	10.0	-	-	-	-	-
Premix grower[3]	-	-	-	-	-	10.0	10.0	10.0	10.0	10.0
Limestone	17.2	16.9	16.1	16.1	16.1	14.3	13.8	13.2	13.2	13.2
Monocalcium phosphate	11.8	12.2	11.5	11.5	11.5	11.1	11.6	10.7	10.7	10.7
Sodium bicarbonate	1.62	1.00	0.55	0.55	0.58	1.33	1.91	0.16	0.16	0.21
NaCl	1.54	1.90	1.88	1.88	1.87	1.47	1.94	1.57	1.57	1.56
L-Lysine HCl	1.84	1.26	1.30	1.30	1.31	2.09	1.40	1.67	1.67	1.69
DL-Methionine	2.23	2.42	2.11	2.11	2.10	2.11	2.40	1.88	1.88	1.86
L-Threonine	0.51	0.49	0.52	0.52	0.52	0.28	0.25	0.28	0.28	0.28
Hylon VII[4]	-	150.0	-	-	-	-	150.0	-	-	-
Allzyme SSF[5]	-	-	-	0.2	-	-	-	-	0.2	-
Capric acid (C10)[6]	-	-	-	-	3.0	-	-	-	-	3.0
Lauric acid (C12)[6]	-	-	-	-	27.0	-	-	-	-	27.0
Calculated chemical composition, g·kg^{-1}										
Crude ash	58	57	57	57	57	52	54	51	51	51
AME_n [7] (poultry), kcal·kg^{-1}	2971	3032	3246	3246	3220	3050	3120	3141	3141	3110
AME_n (broiler), kcal·kg^{-1}	2825	2825	2825	2825	2825	2900	2900	2900	2900	2900

AFD Lys[8]	10.48	10.48	10.48	10.48	10.48	9.87	9.87	9.87	9.87	9.87
AFD Met	5.24	5.31	5.03	5.03	5.03	4.80	4.95	4.52	4.52	4.51
AFD Met + Cys	7.86	7.86	7.86	7.86	7.86	7.40	7.40	7.40	7.40	7.40
AFD Thr	6.79	6.79	6.79	6.79	6.79	6.22	6.22	6.22	6.22	6.22
AFD Trp	2.02	2.11	2.22	2.22	2.23	1.96	2.05	2.16	2.16	2.16
AFD Ile	7.49	7.60	7.54	7.54	7.54	7.05	7.16	7.06	7.06	7.05
AFD Arg	12.45	12.80	12.41	12.41	12.39	11.80	12.36	11.62	11.62	11.60
AFD Val	8.38	8.38	8.38	8.38	8.38	7.90	7.90	7.90	7.90	7.90
Linoleic acid	16.1	12.1	34.9	34.9	24.9	18.5	17.0	23.1	23.1	16.3
Na	1.6	1.6	1.6	1.6	1.6	1.4	1.4	1.4	1.4	1.4
K	7.1	7.0	7.0	7.0	7.0	7.0	8.1	6.5	6.5	6.5
Cl	2.0	2.0	2.0	2.0	2.0	1.8	1.8	1.8	1.8	1.8
Analyzed composition, $g \cdot kg^{-1}$										
CP	201	203	211	217	213	190	190	208	203	203
Crude fat	34	28	102	106	76	48	50	85	83	56
Crude fiber	19	17	28	25	23	15	14	22	26	24
DM	893	898	906	912	897	886	890	905	898	891
Ca	9.8	10.0	10.3	10.0	9.5	8.1	8.2	8.2	8.2	7.9
P	6.6	6.5	6.7	6.8	6.6	6.1	6.0	6.5	6.5	6.2

[1]MCFA = medium-chain fatty acid.

[2]Contributed per kg diet: riboflavin, 4.5 mg; niacinamide, 40 mg; D-pantothenic acid, 9 mg; choline chloride, 500 mg; cyanocobalamin, 20 µg; vitamin E (DL-α-tocopheryl acetate), 30 mg; menadione, 2.3 mg; vitamin A (retinyl-acetate), 12,500 IU; cholecalciferol, 5,000 IU; biotin, 0.1 mg; folic acid, 0.5 mg; $FeSO_4 \cdot H2O$, 147 mg; MnO_2, 100 mg; $CuSO_4 \cdot 5H_2O$, 40 mg; $ZnSO_4 \cdot H_2O$, 143 mg; Na_2SeO_3, 0.5 mg; KI, 2 mg; antioxidant (oxytrap PXN), 125 mg.

[3]Contributed per kg diet: riboflavin, 4.5 mg; niacinamide, 30 mg; D-pantothenic acid, 8 mg; choline chloride, 400 mg; cyanocobalamin, 20 µg; vitamin E (DL-α-tocopheryl acetate), 20 mg; menadione, 2.3 mg; vitamin A (retinyl-acetate), 10,000 IU; cholecalciferol, 2,000 IU; biotin, 50 µg; folic acid, 0.5 mg; $FeSO_4 \cdot H2O$, 147 mg; MnO_2, 100 mg; $CuSO_4 \cdot 5H_2O$, 40 mg; $ZnSO_4 \cdot H_2O$, 143 mg; Na_2SeO_3, 0.5 mg; KI, 1.9 mg; antioxidant (oxytrap PXN; Trouw Nutrition, Putten, the Netherlands), 125 mg.

[4]70% amylose corn starch. National Starch, Manchester, UK.

[5]Allzyme SSF, Alltech Inc., Nicolasville, KY.

[6]Chempri, Raamsdonkveer, the Netherlands.

[7]Calculated according to CVB (2006).

[8]AFD = apparent fecal digestible calculated according to CVB (2006).

Microarray Construction and Analysis

The chicken intestinal microarray used in this study was constructed as described previously for the oral microbiota array for children (Crielaard et al., 2011) and the human intestinal microbiota array (Ladirat et al., 2013). For the chicken intestinal microarray, oligonucleotides based on the 16S rRNA gene sequences and *Enterobacteriaceae hsp*60 gene sequences were used. The 16S rRNA gene sequences were defined based on sequences of the small subunit ribosomal RNA gene V5-V6 region derived from individual chicken ileal and cecal samples collected from 454 pyrosequencing of PCR amplicon libraries using ARB software (Ludwig et al., 2004) and a RDP database (Cole et al., 2007). Species-specific probes, probes representing groups of species (at higher taxonomic levels: genus, family, order) and a general eubacterial probe were selected. Short oligonucleotide sequences were selected with a melting temperature of 60°C according to the Wallace rule (Wallace et al., 1979). All oligonucleotides to be spotted on the arrays contained a 5' NH_2-C_6 extension for improved attachment on the microarray surface. Oligonucleotides were dissolved individually in a 50 m*M* phosphate buffer (pH 9) giving a final concentration of 25 µ*M* oligonucleotide solution. By using TeleChem SMP3 quill pins in an SDDC-2 Eurogridder (BioRad, Veenendaal, the Netherlands) 0.03 pmol of each oligonucleotide is spotted on the CodeLink slide (Surmodics Inc., Eden Prairie, MN), giving a spot of 100 µ*M* in diameter. After spotting microarray slides were cross-linked according to the manufacturer's instructions and stored at room temperature. The microarray contains 370 different probes covering the various bacterial genera, groups of species, and various individual species naturally present in the microbiota of the chicken gut. The construction of this microarray followed a similar method previously described for the human intestinal microarray (Ladirat et al., 2013). The performance of the intestinal microarray was evaluated according to the criteria described and evaluated by Crielaard et al. (2011).

The DNA for microarray analysis was isolated from 200 mg of ileal digesta material. As a starting point, ileal digesta samples were homogenized using zirconium beads (0.1 mm) and phenol in a BeadBeater (BioSpec Products Inc., Lab Services B.V., Breda, the Netherlands) set for 2 min. After cooling on ice, the samples were centrifuged and the DNA isolated from the supernatant using a commercial DNA isolation kit (Agowa, LGC Genomics, Berlin, Germany), following the manufacturer's instructions. Bacterial ribosomal sequences were universally amplified and labeled in a PCR reaction containing the combination of 2 forward primers 16s-8-F (5'-AGAGTTTGATCHTGGYTCAG-3') and 16s-8-BIF (5'-

TGGCTCAGGATGAACGCTG-3') both having a 5' phosphor modification in combination with the reverse primer 16s-1061-R (5'-TCACGRCACGAGCTGACGAC-3') containing a 5'-C6 Cy3 modification. In the same PCR reaction, the *Enterobacteriaceae hsp*60 gene sequences were amplified using the forward primer Entero(Hsp60)-F (5'-GGTAGAAGAAGGCGTGGTTTGC-3') having a 5' phosphor modification and reverse primer Entero(Hsp60)-R (5'-ATGCATTCGGTGGTGATCATCAG-3') containing a 5'-C6 Cy3 modification.

After amplification, the PCR amplicons were purified by passing through a Sephadex column (Autoseq G-50, GE Healthcare Biosciences, Diegem, Belgium) following the manufacturer's instructions. The samples were dried by speed-vacuum centrifugation, re-suspended on a mixture of 0.5 µL Strandase enzyme (NovaGen, Merck Chemicals B.V., Amsterdam, the Netherlands), 1 µL Strandase buffer and 6.5 µL of water was added and incubated for 30 min at 37°C, followed by inactivation of the enzyme during 10 min at 75°C. Next, 12 µL of water was added to the solution, DNA was purified again with an Autoseq G-50 column and dried. The concentration and integrity of the PCR product (1,053 bp) and single-stranded DNA were analyzed by electrophoresis on an ethidium bromide-stained agarose gel. The single-stranded sequences were dissolved in 40 µL of hybridization buffer (Easyhyb, Roche Diagnostics Netherlands B.V., Almere, the Netherlands) and the solution was heated for 2 min in a thermoblock at 95°C. Oligonucleotide microarray slides were covered with hybridization mix and coverslips and were placed in an incubator shaker during 4 h (37°C and 170 rpm). After hybridization, the slides were washed with 1 x SSC, 0.2% SDS, then 0.5 x SSC at 37°C. Two additional washing steps were conducted at room temperature: 5 min of 0.2 x SSC at 280 rpm. The slides were dried with N_2 flow and scanned with a ScanArray Express 4000 Scanner at 10-µm pixel size (Perkin-Elmer Netherlands B.V., Groningen, the Netherlands).

Analysis of Microarray Data

Imagene 5.6 software (BioDiscovery, Hawthorne, CA) was used to analyze the results of the microarray analysis. Signals were quantified by calculating the means of all pixel values of each spot and calculating the local background around each spot. Spots with signal to background ratio >2 were selected for further analysis. To discard data resulting from

technical noise, the minimal number of observations with results more than 2 times above its local background for each spot was set to 10. The spots were scaled based on the highest individual signal to background ratio for each spot where the fluorescent signal divided by the background fluorescent signal was set to 150. The obtained data matrix was used for hierarchical clustering as an unsupervised tool to observe overall similarities between individual samples and supervised biostatistical procedure (Saeed et al., 2003) analysis to identify markers significantly different between predefined groups (TIGR MeV, JCVI, Rockville, MD). Euclidian distance was used as the metric distance and when appropriate complete linkage clustering was performed.

Statistical Analysis

For comparison of the different treatments, all data were subjected to mixed model analysis using the PROC MIXED procedure in JMP (version 9.2, 2008, SAS Institute Inc., Cary, NC) according to the following statistical model:

$$Y_{ij} = \mu + \tau_i + B_j + \varepsilon_{ij}$$

where Y_{ij} = specific trait measured for each experimental unit, μ = overall mean for the specific trait, τ_ι = fixed effect of treatment (i = I, II, III, ..., V); B_j = random block effect (j = 1, 2, 3, ..., 6), and ε_{ij} = residual error term. Cage was the experimental unit. Least square means were separated using Tukey's honestly significant difference. Least square means were assumed to be significantly different based on the probability of $P < 0.05$ unless another probability value is stated.

RESULTS

Performance

Mortality rate was high in present experiment with 8.6%, although no significant differences between dietary treatments were found (data not shown). No explanation for the high mortality was identified, and no clinical signs of disease were observed, although it may be indicative of some subclinical disease challenge during the experiment. There was a significant effect of dietary treatment on technical performance traits. The ST diet resulted in a higher ($P < 0.0001$) BW gain from 0 to 13 d of age by 7.1% and feed intake by 7.4% compared with the CO diet (Table 2).

Table 2. Body weight, BW gain, feed intake, and feed conversion ratio of broiler chickens as affected by 5 different diet compositions[1]

Item	Corn	Starch	Wheat	Enzyme	MCFA[2]	Pooled SEM	Model *P*-value
BW 34d, g	1960 [ab]	2063 [a]	1804 [b]	1873 [b]	1929 [ab]	40.2	0.003
BW gain, g							
0 to 13 d	380 [b]	407 [a]	300 [d]	317 [cd]	326 [c]	5.4	<0.0001
13 to 34 d	1540	1617	1464	1517	1563	37.2	0.094
0 to 34 d	1920 [ab]	2024 [a]	1764 [b]	1834 [b]	1890 [ab]	40.2	0.003
Feed intake, g							
0 to 13 d	457 [b]	491 [a]	364 [c]	379 [c]	375 [c]	7.2	<0.0001
13 to 34 d	2544 [ab]	2728 [a]	2317 [b]	2367 [b]	2349 [b]	55.0	0.0001
0 to 34d	3001 [a]	3220 [a]	2681 [b]	2745 [b]	2724 [b]	59.5	<0.0001
Feed conversion ratio							
0 to 13 d	1.202 [a]	1.209 [a]	1.213 [a]	1.196 [a]	1.148 [b]	0.011	0.003
13 to 34 d	1.652 [a]	1.688 [a]	1.583 [b]	1.561 [b]	1.503 [c]	0.014	<0.0001
0 to 34 d	1.563 [ab]	1.591 [a]	1.520 [bc]	1.498 [c]	1.441 [d]	0.010	<0.0001
Corrected[3]	1.516 [a]	1.513 [a]	1.520 [a]	1.477 [ab]	1.404 [b]	0.017	0.001

[a–d]Effect means within rows with no common superscript differ significantly ($P < 0.05$).
[1]n = 6 replicates for all treatments.
[2]MCFA = medium-chain fatty acids; Chempri, Raamsdonkveer, the Netherlands.
[3]Corrected to a final BW of 1,804 g, with a 0.03-point correction for each 100-g weight difference.

There was no difference in FCR from 0 to 13 d of age between the CO and ST diet. From 13 to 34 d and from 0 to 34 d of age there were no differences in BW gain, feed intake, and FCR between the CO and ST diet. Adding the enzyme complex to the WHE diet did not improve BW gain, feed intake, or FCR during any time period (Table 2). Replacing animal fat and soybean oil in the WHE diet with MCFA (and 4.4 g·kg^{-1} wheat) improved ($P < 0.0001$) BW gain from 0 to 13 d of age by 8.7%. No difference in feed intake was observed between the MCFA and the WHE diet. Feed conversion ratio of the MCFA diet improved by 5.4% ($P = 0.003$) from 0 to 13 d of age and by 5.2% ($P < 0.0001$) from 0 to 34 d of age. Feed intake from 0 to 34 d of age was higher for the CO and ST diets compared with the WHE, ENZ, and MCFA diets (Table 2). Feed conversion ratio was lower for the ENZ and MCFA diets compared with the WHE, CO, and ST diets. However, when corrected for final BW, the FCR of the MCFA diet was lower compared with the other 4 diets.

Microbiota

Analysis of the ileal digesta microbiota of the different birds by using the intestinal microarray in this study provides an insight into the diversity of the microbiota between individual birds. As general eubacterial probes were present on the chip, the array data provide a semiquantitative insight into the presence of specific taxonomical entities in the microbiota sampled from the birds. A total of 321 probes, out of the 370, showed a positive signal when analyzing the microbial composition of the ileal digesta. Hierarchical clustering of the ileal digesta microbiota of the individual bird samples did not show a full clustering based on the type of diet (Figure 1). The variation between individual birds per dietary treatment was more pronounced than variation caused by feed composition, with the exception of the digesta microbiota of the birds fed the MCFA diet. Figure 1 shows a clear clustering of the microbiota composition of 10 from the 16 individual birds fed the MCFA diet compared with the other 4 diets.

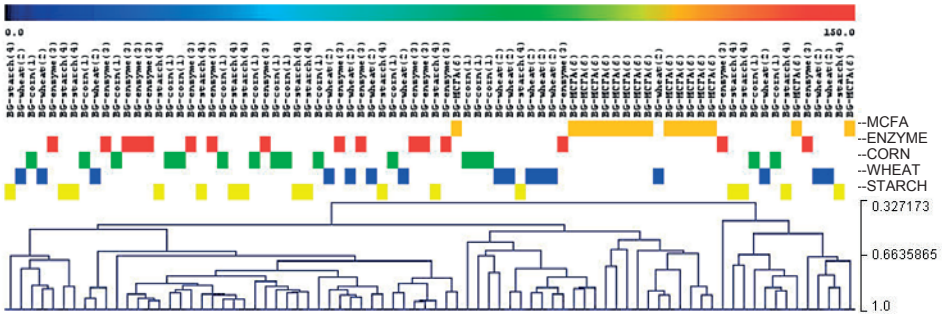

Figure 1. Hierarchical clustering of microarray data obtained from 80 ileal digesta samples of which groups of 16 samples are each representing individuals from a specific diet group of birds. The feed conditions are presented in the right margin with the similarity coefficient given in the lower right margin. The scale shown at the top bar above the figure indicates the signal intensity of the bacterial DNA hybridized to the probes on the chip. MCFA = medium-chain fatty acids.

Additional significance analysis of microarrays (Saeed et al., 2003) of the effect of the MCFA diet on the ileal digesta microbiota compared with the other diets is shown in Figure 2. The MCFA diet suppressed many species belonging to the phylum of the *Firmicutes* including *Lactobacillus* species and species belonging to the family *Micrococcaceae* and *Enterococcaceae*. In contrast, the MCFA diet promoted species belonging to the genus *Enterobacteriaceae* and specific *Lactobacillus* species compared with the other 4 diets.

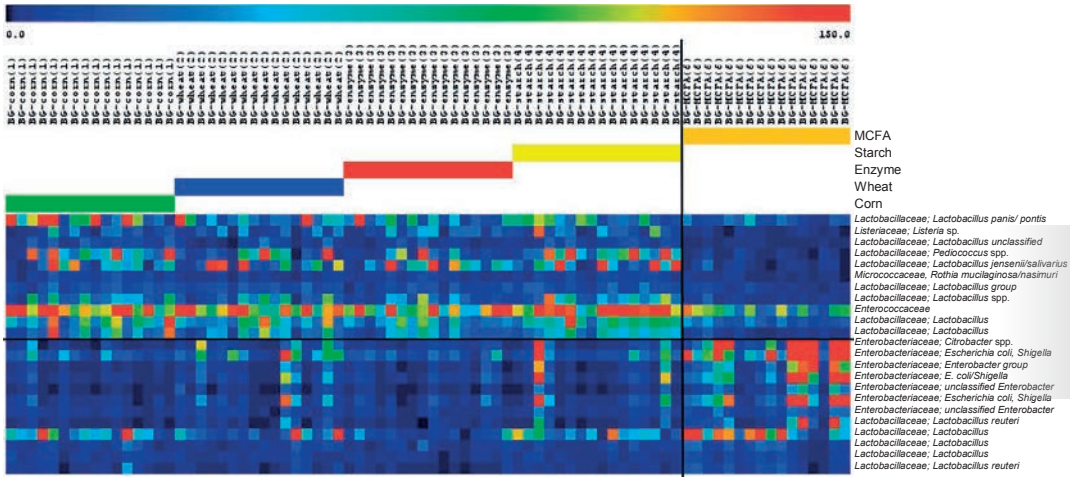

Figure 2. Significance analysis of microarrays for 80 ileal digesta microbiota samples of which 16 individuals belong to the medium-chain fatty acid (MCFA)-based diet group and 64 individual birds to the other 4 diet groups. The heat map shows those probes representing 16S rRNA gene sequences that were significantly different between the 2 groups, where each column represents one individual bird. The scale shown in the top bar of the figure indicates the signal intensity of the bacterial DNA hybridized to the probes on the chip. Oligonucleotide sequence identifiers in the right margins represent the probes including their representing bacterial species.

Three bacterial groups were promoted in the ileal digesta of birds fed the WHE compared with the CO diet (Figure 3): *Staphylococcus* spp., including the species *Staphylococcus aureus,* and *Brenneria* species (*Enterobacteriaceae*).

Feeding the ENZ diet significantly suppressed several bacterial groups compared with the WHE diet: *Brachybacterium* spp., including the species *Brachybacterium muris* and *Brachybbacterium rhamnosum, Brevibacterium* spp., *Brenneria* spp., *Lachnospiraceae, Corynebacterium* spp., including the species *Corynebacterium lipophiloflavum* and *Corynebacterium tuberculostearicum,* and *Staphylococcus aureus* (Figure 4).

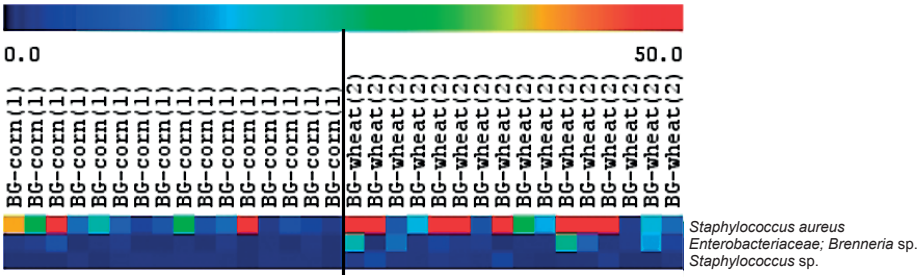

Figure 3. Significance analysis of microarrays of ileal digesta microbiota of 16 individual birds fed the corn diet and 16 individual birds fed the wheat diet. The heat map shows those probes representing 16S rRNA gene sequences that were significantly different between the 2 groups, where each column represents one individual bird. The scale shown in the top bar above the figure indicates the signal intensity of the bacterial DNA hybridized to the probes on the chip. Oligonucleotide sequence identifiers in the right margins represent the probes including their representing bacterial species.

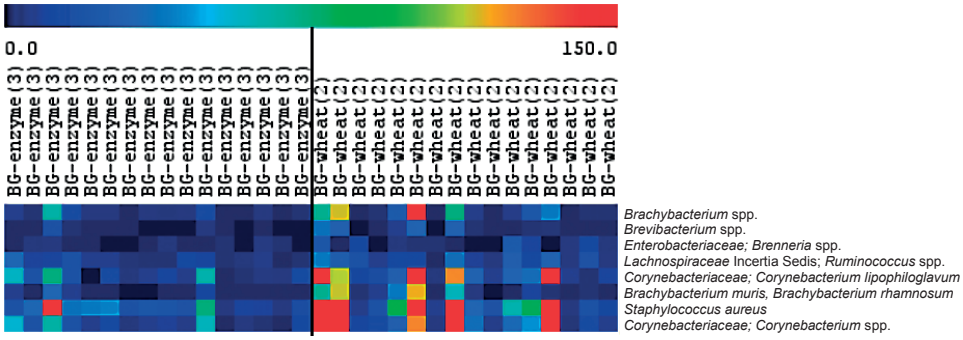

Figure 4. Significance analysis of microarrays of ileal digesta microbiota of 16 individual birds fed the enzyme diet and 16 individual birds fed the wheat diet. The heat map shows those probes representing 16S rRNA gene sequences that were significantly different between the 2 groups, where each column represents one individual bird. The scale shown in the top bar above the figure indicates the signal intensity of the bacterial DNA hybridized to the probes on the chip. Oligonucleotide sequence identifiers in the right margins represent the probes including their representing bacterial species.

Digesta pH

The pH of the digesta in the ileum was not affected (P = 0.88) by diet composition (Table 3). Ceca digesta pH was lower (P = 0.005) for the MCFA diet compared with the CO and ST diet, and not different between the different wheat-based diets.

Excreta

Excreta DM content at 34 d of age was not affected ($P = 0.58$) by any of the dietary treatments (Table 3). Also, excreta pH at 34 d of age was not affected by dietary composition ($P = 0.68$).

Table 3. Excreta DM and pH of the ileum and ceca digesta at 34 d of age of broiler chickens as affected by 5 different diet compositions[1]

Item	Corn	Starch	Wheat	Enzyme	MCFA[2]	Pooled SEM	Model *P*-value
Excreta DM, %	23.5	29.0	25.8	25.7	24.7	2.02	0.423
pH							
Ileum	6.40	6.39	6.41	6.51	6.21	0.20	0.876
Ceca	6.63 [a]	6.68 [a]	6.44 [ab]	6.38 [ab]	6.19 [b]	0.09	0.005
Excreta	5.47	5.36	5.33	5.43	5.26	0.11	0.678

[a–b]Effect means within rows with no common superscript differ significantly ($P < 0.05$).
[1]n = 6 replicates for all treatments. pH value is an average of 3 birds per cage.
[2]MCFA = medium-chain fatty acids; Chempri, Raamsdonkveer, the Netherlands.

DISCUSSION

The MCFA diet showed the most clear and significant effect on the GIT microbiota of the ileum. The other diets used in the present study did not affect ileal digesta microbiota composition notably. The MCFA diet suppressed and promoted several bacterial species compared with the 4 other diets. The suppressed bacteria included many gram-positive bacteria belonging to the phylum of the *Firmicutes* including several *Lactobacillus* species and species belonging to the family *Micrococcaceae* and *Enterococcaceae* (Figure 2). The promoted species belonged mainly to the gram-negative family *Enterobacteriaceae* and some minor species at a low level of detection belonging to the genus *Lactobacillus*. The results indicate that minor species belonging to the genus *Lactobacillus* are promoted in the presence of MCFA while prominent *Lactobacillus* species are suppressed. The changes in *Lactobacillus* species coincides with a high abundance (70%) of *Lactobacillus* presents in the broiler ileum, whereas *Enterococcaceae* have previously been detected at a level of 6.4% (Lu et al., 2003).

The suppression of several gram-positive bacteria species and groups of species is in line with findings of Kabara et al. (1972) and Skřivanová et al. (2005), who found higher fatty acid sensitivity in gram-positive bacteria compared with gram-negative bacteria.

Entercococcus hirae was previously detected in poultry intestines by Farrow and Collins (1985) and held responsible for growth depression, higher FCR, and increased mortality in broilers (Chadfield et al., 2005). The reduction in *Lactobacillus* spp. at the ileum induced by the MCFA diet could result in a reduced bile salt deconjugation by these bacteria. Guban et al. (2006) found improved BW gain and FCR in broilers fed antimicrobial drugs (bacitracin and monensin), consistent with a reduction in *Lactobacillus salivarius* responsible for deconjugation of bile salts in vitro. Additionally, Torok et al. (2011) found an association between the presence of a specific taxonomic unit, potentially representing *L. salivarius*, *L. avaiarius*, and *L. crispatus*, and reduced production performance in broilers. The MCFA diet could exert its effect via different pathways. Medium-chain fatty acids can have a direct inhibiting effect on bacteria (Kabara et al., 1972; Skřivanová et al., 2005), or indirect effects via endogenous systems such as the immune system (Lee et al., 2001).

Substrate limitation could increase competition between microbial species and thereby change the composition of the microbiota, a process called competitive exclusion. A change in pH value could be indicative of changed production of short-chain fatty acid by bacteria during fermentation (Engberg et al., 2004). Although the ST diet was intended to increase substrate availability for the microbiota in the distal GIT and, thereby, possibly affect digesta pH, the pH of the ileal and cecal digesta of birds fed the ST diet was similar to the CO diet (Table 3). Furthermore, no changes in ileal digesta microbiota between the CO and the ST diets were observed in this present study. In agreement with our findings, Rehman *et al.* (2008) found no effect of inulin on microbial community composition in the ceca. Possibly, the short transit time of the digesta limits the time of exposure of starch molecules to the microbiota and thus reduces the capacity of the microbiota to ferment resistant starch (Thompson et al., 2008; Choct et al., 2010). Adding an NSP enzyme complex to the wheat-based diet reduced the signal level of several microbial species measured by microarray in the ileal digesta compared with the WHE diet (Figure 4), indicating a reduction of their presence in the ENZ diet. This finding seems to confirm that enzymes reduce substrate availability in the distal GIT for some bacterial species. However, this could not be linked to changes in ileal or cecal digesta pH observed between the WHE and ENZ diets. The reduced presence of specific bacterial groups in the ileal digesta when the ENZ diet was fed is consistent with the findings of Owens et al. (2008), who reported reduced ileal digesta *Lactobacillus* spp. and coliform counts after xylanase supplementation. In contrast, Vahjen et al. (1998) found increased mucosa tissue-associated *Lactobacillus* species and reduced enterobacterial and

gram-positive coccal counts, but no differences in luminal counts when a xylanase was fed. Response differences to enzymes may be related to dietary ingredients used, digestibility of the diet, bird strain, and age as reviewed by Bedford and Cowieson (2012). The hybridization level of *Staphylococcus* spp., including *Staphylococcus aureus,* and *Brenneria* species (*Enterobacteriaceae*) was higher in the ileal digesta of birds fed the WHE compared with the CO diet. Although no quantitative data on the abundance of the species is provided with the technique used, the presence of *Staphylococcus* was previously detected in the ileum at 1% by Lu et al. (2003).

Besides the effect of the MCFA diet on ileal digesta microbiota, the MCFA diet also reduced the pH of the cecal content. The causal relationship between the MCFA diet composition and reduced cecal pH is not completely understood. Possibly, the production of short-chain fatty acids by the microbiota was affected as a consequence of alterations of cecal bacteria composition, leading to a reduction in luminal pH (Buddington, 2001) or the volatile fatty acids produced may affect microbial composition (van der Wielen et al., 2000).

When analyzing the individual microbial profiles, a large variation between individuals within a dietary treatment group was found (Figure 1). This variation in microbiota occurred between individual birds with the same genetics and housed in the same environment. Similarly large individual bird variation was observed by Owens et al. (2008), Torok et al. (2011) and Apajalahti et al. (2001). The source of this individual variation seems to be something other than genetics or dietary in origin, and may be related to other factors such as social hierarchy affecting stress, early life immunological development, and epigenetic factors. As a result, pooling samples of birds from the same experimental unit may therefore mask pronounced differences between individual birds and should be avoided (Bedford and Cowieson, 2012).

Feeding the birds diets containing similar contents of ME and apparent fecal digestible lysine, methionine + cystine, threonine, and valine resulted in similar final BW (Table 2). Feed conversion ratio was numerically reduced by feeding the wheat-based compared with the corn-based diet. However, this cannot be fully attributed to the difference in basal grain, as other ingredient inclusions (e.g., added fat) also changed to facilitate equal ME and AA content diet formulation. The MCFA diet reduced FCR compared with the WHE and ENZ diet. However, this could be related to a numerically lower final BW of the birds fed a wheat-based diet and a subsequent lower maintenance requirement. When correcting the FCR to an

equal final BW, using a correction factor of 0.03 points per 100 g BW, FCR was lower for the MCFA diet compared with the other 4 diets. This finding may coincide with the difference in ileal digesta microbiota caused by the different dietary compositions and confirms the findings of Farrow and Collins (1985), Guban et al. (2006), and Torok et al. (2011). These authors found a positive relationship between reduced numbers of *Entercococcus hirae* and a specific taxonomic unit, potentially representing several *Lactobacillus* species, and feed efficiency in broilers. It should, however, be noted that the changes in ileal bacteria profile cannot be attributed to MCFA alone, but could also be a result of other changes in the diet composition (e.g., 0.65% more wheat and reduced animal fat and soya oil content) compared with the WHE diet. These changes were necessary to keep an equal ME and amino acid content between diets.

It was hypothesized that a change in nutrient load in the ileum could increase osmotic pressure in the distal GIT, resulting in an increased water output (Choct and Annison, 1992). However, excreta DM was not affected by any of the diets in current experiment (Table 3). This implies that the changes found in the ileal digesta microbiota in this experiment did not affect excreta moisture output. These results are in line with findings by Dänicke et al. (1999) and Engberg et al. (2000), who reported no differences in digesta DM with xylanase or ionophore antibiotic supplementation, respectively, even though ileal and cecal microbial differences were present.

In summary, none of the diets varying in ingredients (cereal, enzyme) that can affect the GIT microbiota and used in the present study notably changed ileal digesta bacterial composition, except for the MCFA-containing diet. The latter suppressed the gram-positive *Firmicutes* including *Lactobacillus* species and species belonging to the family *Enterococcaceae* and *Micrococcaceae*. The changes in ileal microbiota composition coincided with improved feed efficiency but had no effect on excreta DM content. The array approach can be seen as a holistic approach to identify significant changes in the microbiota as a consequence of dietary composition differences. The identification of species correlating with, in this case, specific diet conditions provides a further basis for more “reductionistic” studies to precisely identify relevant changes in the microbiota by techniques such as culturing specific species or quantitatively detecting them by using specific qPCR detection approaches. Ultimately that can be a basis for causality studies. Such more in-depth studies regarding the relationship of specific bacteria species with production performance are required to define healthy changes in the microbiota profile of broilers.

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Chapter 3

Moisture content in broiler excreta is influenced by excreta nutrient contents

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ABSTRACT

High litter moisture content, often referred to as wet litter, is a major problem in poultry production. Wet litter is often related to poor management, diseases, and digestive problems. In this experiment, the objective was to study the relationship between nutrient content and the moisture content of the excreta of broilers. A dataset containing 351 observations was built and contained the nutrient contents data including moisture content of excreta samples collected in 8 different broiler feeding trials. A biological based model approach was used to create a model with 10 and another one with 14 variables that may explain the excreta moisture level response. Subsequently, these models were compared with a statistical model that was built automatically and adjusted only if this improved the biological model. The R^2 of the 10 variable model was 0.54, in which Zn content and the interaction of NDF × K and Ca × P content were negatively associated with excreta moisture. Sodium, P, and Ca content and the interaction between content of NDF × Na were positively associated with excreta moisture. The R^2 of the 14 variable model was 0.58, in which Zn and K content and the interaction of NDF × protein and Ca × P content were negatively associated with excreta moisture, and Na, protein, P, and Ca content and the interactions in contents of NDF × Na, NDF × Zn, and K × Cu were positively associated with excreta moisture content. In conclusion, the models confirmed the effect of Na, protein, P, and Ca on excreta moisture content. Furthermore, hitherto unknown nutrient interactions that contribute to excreta moisture level were identified. As excreta levels of most nutrients can be manipulated by adjusting dietary nutrient levels, dietary formulation can be adjusted with the findings of this analysis to change levels of excreted nutrients and, consequently, also moisture output.

Key words: broilers, excreta moisture, general linear mixed model, nutrient excretion, wet litter

INTRODUCTION

Broilers are housed on litter, composed of bedding material mixed with feed, feathers, and excreta (Cook et al., 2011). Above a certain litter moisture content, "wet litter" can occur causing more ammonia to be produced and emitted into the air (Groot Koerkamp, 1994), which can negatively affect animal production and welfare (Kristensen and Wathes, 2000).

Fresh poultry droppings contain approximately 80% moisture (Henuk and Dingle, 2003) and significantly contribute to litter moisture content (Groot Koerkamp et al., 1999a). Dietary nutrient composition can affect excreta moisture content through nutrient digestibility and passage rate of digesta through the gastrointestinal tract (**GIT**). Dietary mineral levels and ratios between minerals play an important role in excreta moisture content (Ziaei et al., 2008; Jankowski et al., 2011). Inconsistent effects of trace minerals on excreta moisture have, however, been reported by Nishimuta et al. (2006) and Zhong et al. (2007). A high dietary protein content (Collett, 2012) or insoluble fibers in the diet, which can have a high water binding capacity in the GIT (Chaplin, 2003), can increase the water content of droppings.

The effect of single dietary nutrients on excreta moisture level in poultry has been previously studied (Ferguson et al., 1998b; Smith et al., 2000b; Namroud et al., 2009). Comparing the effects of various nutrients and nutrient interactions in a single statistical analysis on excreta moisture content may provide new insights into the multifactorial problem of wet litter in poultry production. The current study was undertaken to investigate relationships between moisture and nutrient content (CP, NDF, and minerals) of broiler excreta using a linear mixed model analysis to identify hitherto undocumented associations that could potentially be used in dietary strategies for wet litter prevention.

MATERIALS AND METHODS

The experimental methods were approved by the Ethical Committee of the Animal Science Group of Wageningen University and Research Center, Lelystad, the Netherlands.

Animal Experiments

The studies reported here were conducted during 2008 and 2009 at the research facilities of the Cargill Innovation Center Velddriel, Velddriel, the Netherlands. Birds were housed in 2 barns. Barn 1 had cages (100 x 110 cm) with a raised wire floor on top of which a rubber plate was placed and covered with a 2 cm layer of wood shavings. Each cage housed 18 birds and was equipped with 2 adjustable cup drinkers and a feeder that was positioned inside the cage during the first 14 d. From 15 d onward, feed was supplied via a feed trough in front of the cage. Birds were housed in this barn until 35 d of age. Continuous artificial lighting was maintained for 23 h/d throughout the experiments. Temperature, relative humidity, and ventilation were computer controlled with the temperature gradually decreasing by 2.5°C per week, from 34.0°C on the day of arrival (1-d-old chicks) to a final temperature of 20.0°C at the end of the experiment (35 d). Relative humidity was set at 50% throughout the experiments. Barn 2 had cages of 50 x 50 cm, each with 6 birds. Each cage was equipped with 2 adjustable nipple drinkers. During the first 3 d, a feeder was positioned inside the cage. From 4 d onward, feed was supplied via a feeder trough in front of the cage with adjustable access opening and placement height. Birds in this barn were grown until 17 d of age. Continuous artificial lighting was maintained for 23 h/d for the first 3 d of the experiments, 20 h/d between 4 and 7 d, and 18 h/d for the remainder of the experiments. Temperature, relative humidity, and ventilation were computer controlled with the temperature gradually decreasing by 0.5°C per day, from 33.0°C on the day of arrival (1-d-old chicks) to a final temperature of 26.1°C at the end of the experiment (17 d). Room temperature was recorded continuously using data loggers, and relative humidity was set at 50% throughout the experiments.

Excreta collection during the trials was achieved using an excreta collection box, which was placed on top of the litter and facilitated pure excreta sampling without litter. At the end of the experiment litter from the cages was removed and, subsequently, excreta were collected from metal plates that had been placed underneath the cages. All excreta were collected on a cage basis and feathers and feed particles were carefully removed. The excreta samples were collected during 8 different trials that are briefly described in Table 1.

Table 1. Overview of the 8 trials from which excreta samples were collected and analyzed

Trial	Year	Trial objective	Bird age at collection, d	No. of diets	Distinguishing dietary factors	Relation to excreta quality
1	2009	Emulsifiers and cereal inclusion in relation to digestibility	17	24	Additives	Changed nutrient digestibility
2	2009	Feedstuff in relation to microbiota	6, 35	12	Crude fat, crude fiber, NDF, P, and K	Relation of microbiota with excreta quality
3	2008	Additives and cereal in relation to excreta quality	35	12	Crude fat, crude fiber, NDF, and K	Ammonia binding additives and fibers
4	2008	Dietary protein level in relation to growth	35	6	CP, crude fat, crude fiber, NDF, Ca, P, K, and Cl	Dietary protein content
5	2008	Feedstuff form and protein quality in relation to growth	35	6	CP, crude fat, Ca, P, Na, K, and Cl	Protein quality and GIT[1] stimulation due to structure
6	2008	Starch resistance in relation to feed efficiency	35	14	Crude fat and starch	Starch fermentation by increased resistance
7	2008	Feedstuff form and NSP[2] enzymes in relation to feed efficiency	35	12	CP, crude fat, crude fiber, NDF, P, and K	Changed nutrient digestibility and GIT[1] stimulation due to structure
8	2009	Mineral levels in relation to excreta quality	14, 28, 35	24	Range of Ca, P, Na, and Cl	Mineral level and ratios

[1]GIT = gastrointestinal tract.
[2]NSP = nonstarch polysaccharides.

Experimental Feedstuffs

A total of 110 different dietary formulations were fed throughout the 8 trials. Replications differed per dietary formulation and ranged from 1 to 12. However, as treatment was not an effect of interest, this was not considered a limitation. In all trials feed and water were provided ad libitum. There was a wide range of dietary nutrient compositions tested. Table 2 shows the minimum, maximum, median, and average value of the dietary nutrients fed.

Table 2. Range of dietary nutrients fed over the 8 different trials where excreta was collected (n = 354)

Item	n	Mean	Minimum	Maximum	Median
AME (broiler), kcal·kg^{-1}, [1]	354	2,868	2,750	3,246	2,850
CP, g·kg^{-1}	354	194	175	245	194
Crude fat, g·kg^{-1}	354	78	28	115	80
Crude fiber, g·kg^{-1}	354	30	22	60	29
NDF, g·kg^{-1}	330	102	79	180	100
Ash, g·kg^{-1}	354	57	41	81	56
DM, g·kg^{-1}	354	881	875	894	880
Ca, g·kg^{-1}	354	8.3	4.3	15	8.0
Cl, g·kg^{-1}	354	1.8	1.5	4.4	1.6
Cu, mg·kg^{-1}	330	20	15	27	20
Fe, mg·kg^{-1}	330	170	124	347	166
K, g·kg^{-1}	354	8.2	6.5	9.7	8.3
Mg, g·kg^{-1}	330	1.5	1.3	2.7	1.5
Mn, mg·kg^{-1}	330	84	75	101	84
Na, g·kg^{-1}	354	1.5	1.4	3.3	1.4
P, g·kg^{-1}	354	6.6	4.7	10	6.5
Zn, mg·kg^{-1}	330	81	75	102	80

[1]AME = Apparent metabolizable energy. Calculated according to CVB (2006).

Excreta Database

To analyze the data from the trials, a database was developed consisting of the wet chemically analyses of the excreta samples. The samples were analyzed for DM (Gravimetry, ISO 6496; Memmert UNB 500, Memmert GmbH, Schwabach, Germany), Ca, Cu, Fe, K, Mg, Mn, Na, Zn, P (ICP-AES, ISO 27085:2009; Thermo Iris Intrepid II XSP Duo, Thermo Scientific Inc., Waltham, MA), CP (Combustion, ISO 16634; Rapid N Cube, Elementar GmbH, Hanau, Germany), and NDF (ANKOM Model 200, filter bag technique “method 6” with filter bag type 57, ANKOM Technology, Macedon, NY). Nondetergent fiber measures

lignin, hemicellulose, and cellulose as major components (van Soest et al., 1991) and was used as an indicator of insoluble fiber content. The content of soluble fibers has not been analyzed in the samples. Before statistical analysis, all excreta nutrient content were expressed on a DM basis.

The database contained 351 observations originating from 2008 (n = 178) and 2009 (n = 173) collected from barn 1 (n = 327) and barn 2 (n = 24). The samples were collected at 6 (n = 6), 17 (n = 24), 28 (n = 36), and 35 d of age (n = 285). Most samples originated from Ross 308 male birds (n = 291) although some samples were obtained from Cobb 500 (n = 24) and Hubbard Flex (n = 36) male birds. All birds originated from the same commercial hatchery (Lunteren, the Netherlands). Each trial and treatment combination was numbered with a unique code. Table 3 presents the number of observations and the minimum, maximum, median, and average value of the excreta nutrients.

Table 3. Range in excreta nutrients included in the database, corrected for DM content of the sample (n = 354)

Item	n	Mean	Minimum	Maximum	Median
DM	348	26	17	35	27
Moisture	348	74	65	83	73
CP	350	30	22	43	30
NDF	351	23	6.4	36	25
Ca	349	1.9	0.7	4.1	1.8
K	351	2.3	1.6	2.8	2.4
Mg	351	0.5	0.4	0.8	0.5
Na	350	0.3	0.2	1.1	0.1
P	351	1.5	0.9	2.8	1.4
Cu	350	0.006	0.004	0.010	0.006
Fe	351	0.068	0.045	0.110	0.065
Mn	351	0.030	0.022	0.052	0.029
Zn	350	0.063	0.020	0.181	0.060

Statistical Analysis

Data from the 8 trials were assembled and organized in such a way that for each sample the data for similar independent and dependent variables were listed in columns and trial identification was retained. Additionally, squared terms for all predictor variables were calculated as were all first order interaction terms. The final data set of potential predictor variables included all main effects and their squared terms and first order interactions which were considered nutritionally relevant. The total number of variables offered for inclusion in

the final biological models was 78. This master data set, containing all 78 potential variables, was used for a first screening of potential variables for the statistical models. A subset of 48 potential predictor variables was subsequently used in the construction of the statistical linear models - the reduced dataset.

For the biological models, general linear mixed models, using the PROC MIXED procedure of SAS (version 9.2, 2008, SAS Institute Inc., Cary, NC), were defined following the general form $Y = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\boldsymbol{u} + \mathbf{e}$, where **X** is the known fixed effects design matrix, **ß** is the vector of unknown fixed effects parameters, **Z** is the known random effects design matrix, and **u** and **e** are random effect vectors. Investigation of the 78 variables to be included in the final design matrix of fixed effects (**X**) was performed by identifying their specific contribution to the performance of the prediction equation on the basis of several factors (Hocking, 1976). These factors included: *i*) the significance of the independent variable in the mixed model ($P < 0.10$), *ii*) the contribution of the variable to the log likelihood coefficient of determination as defined by Kramer (2005) for a mixed model, *iii*) the biological importance of the variable as defined by current nutritional understanding, and *iv*) the relationship of the factor to others already included in the reduced model. A cut-off value of $P < 0.10$ was used to not exclude any trends that may be biologically still relevant. To prevent overfitting of the reduced model (Babyak, 2004) and limit exposure to problems arising from the inclusion of too many variables a cap on the total number of predictor variables was arbitrary set at 10 and a second at 14 based on R^2 improvement as shown in Fig. 1.

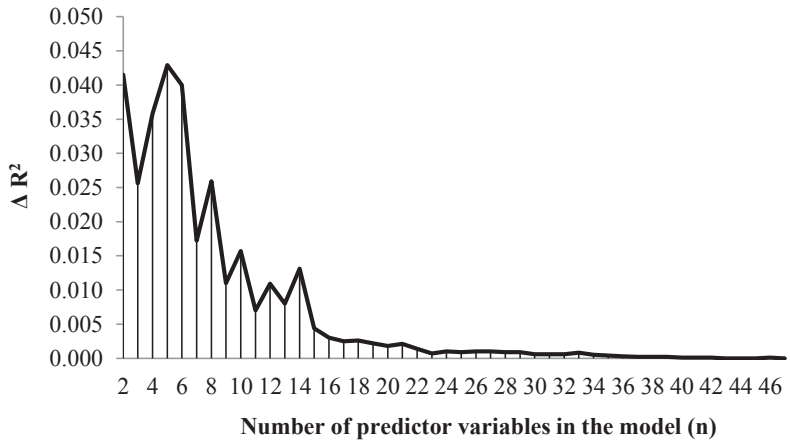

Figure 1. The R^2 improvement per addition of 1 predictor variable to the statistical model explaining excreta moisture.

The principal objective was to provide a reduced model that permitted a clear understanding of relevant nutritional and biological relationships and interactions. The model, however, should not be overly complex. As is the case when any such predictor models are constructed, the inclusion or exclusion of certain predictor variables is somewhat subjective especially when observing limits placed on the total number of terms to be included in the final models.

A third model that was constructed purely on an objective basis of factor significance ($P < 0.05$) and the maximization of the coefficient of determination was completed after the approach detailed by Tilea et al. (2012). In this procedure all potential independent variables were recursively tested for their contribution to a reduced model with 1 to n-1 fixed effect predictor variables. The full model (all 48 predictor variables) was also constructed and this model provided a reference to which all reduced models were compared. The objective recursive models were subsequently used in a comparison to the subjective reduced models to rationalize the process of subjective independent variable selection and check for omissions of variables that had significant contributions as a predictive factor and were ranked according to the variation they explained in the data. As such the final reduced models presented are both statistically meaningful and have biological relevance.

RESULTS

Best 10-variable Biological Model

The least square estimates (the solution to the minimized sum of the squared deviations between predicted and observed values) for the optimal model with 10 variables are presented in Table 4. Random effect variables included in this model were year, trial number, and treatment. The coefficient of determination ($\mathbf{R^2}$) of the model was 0.542. The results show that Zn content and the interactions between contents of NDF × K and Ca × P were negatively associated with excreta moisture, where greater levels of these components resulted in lower excreta moisture levels. Na, P, and Ca content and the interaction between contents of NDF × Na were positively associated with excreta moisture, where greater levels of these components resulted in greater excreta moisture levels.

Table 4. Least squares estimates and corresponding significance levels for the nutrients explaining the excreta moisture content in the excreta from the 10-variable biological linear mixed model

Item	Least square estimate	SE	*P*-value
Age, d of age			<0.001
6	0	.	
17	7.38	4.56	
28	-2.12	1.50	
35	0.40	1.42	
Zn	171.95	25.56	<0.001
Na^2	17.34	4.46	<0.001
P	6.62	1.08	<0.001
Ca	4.27	0.82	<0.001
NDF × Na	0.74	0.19	<0.001
NDF × K	-0.05	0.03	0.049
Ca × P	-2.07	0.47	<0.001
Na	-32.56	5.54	<0.001
Zn^2	-721.06	136.49	<0.001

Table 5. Least squares estimates and corresponding significance levels for the nutrients explaining the excreta moisture content in the excreta resulting from the 14-variable biological linear mixed model

Item	Least square estimate	SE	*P*-value
Age, d of age			<0.001
6	0	.	
17	3.68	2.51	
28	-6.55	1.78	
35	-4.04	1.73	
K × Cu	140.93	75.26	0.062
Zn	80.48	49.17	0.104
Na^2	15.87	4.34	<0.001
P	5.74	1.04	<0.001
Ca	3.81	0.77	<0.001
NDF × Zn	3.39	1.61	0.036
NDF × Na	1.44	0.27	<0.001
CP	0.60	0.13	<0.001
NDF × CP	-0.02	0.01	<0.001
Ca × P	-1.84	0.44	<0.001
K	-2.13	0.96	0.026
Na	-48.79	6.96	<0.001
Zn^2	-665.42	134.45	<0.001

Best 14-variable Biological Model

The least square estimates for the optimal model with 14 variables are presented in Table 5. Random effect variables included in this model were trial number and treatment. The R^2 of the model was 0.583. The results show that Zn and K content and the interactions between NDF × CP and Ca × P contents were negatively associated with excreta moisture, where greater levels of the components resulted in lower excreta moisture levels, and Na, CP, P, and Ca content and the interactions between NDF × Na, NDF × Zn, and K × Cu content were positively associated with excreta moisture content.

Best 10-variable Automated Statistical Model

The least square estimates for the automated statistical model with 10 variables are presented in Table 6. Because of limitations in the calculation method employed by Tilea et al. (2012), no random effect variables could be included in this model. The R^2 of the model was 0.399. Results show that Zn content and the interactions between NDF × CP, NDF × K, Ca × P, and Na × P content were negatively associated with excreta moisture, where greater levels of the components resulted in lower excreta moisture levels. Crude protein and P^2 content and the interactions between Ca × Mg, NDF × Na, NDF × Zn content were positively associated with excreta moisture, where greater levels of the components resulted in greater excreta moisture levels.

Table 6. Least squares estimates and corresponding significance levels for the nutrients explaining the excreta moisture content in the excreta resulting from the 10-variable automated statistical linear model

Item	Least square estimate	SE	*P*-value
Ca × Mg	7.76	1.06	<0.001
NDF × Zn	5.72	1.26	<0.001
P^2	3.95	0.41	<0.001
NDF × Na	1.89	0.22	<0.001
CP	0.64	0.11	<0.001
NDF × CP	- 0.02	<0.01	<0.001
NDF × K	- 0.11	0.03	<0.001
Ca × P	- 2.27	0.37	<0.001
Na × P	- 27.77	3.85	<0.001
Zn	- 134.30	29.40	<0.001

Best 14-variable Automated Statistical Model

The least square estimates for the automated statistical model with 14 variables are presented in Table 7. No random effect variables could be included in this model because of limitations in the calculation method used by Tilea et al. (2012). The R^2 of the model was 0.438. Results show that Zn, K, Cu^2, and Mg content and the interactions between NDF × CP, Ca × P, and Na × P content were negatively associated with excreta moisture, where greater levels of the components resulted in lower excreta moisture levels. Ca, CP, P^2 content and the interactions in contents of NDF × Na, NDF × Zn, and K × Cu were positively associated with excreta moisture content.

Table 7. Least squares estimates and corresponding significance levels for the nutrients explaining the excreta moisture content in the excreta resulting from the 14-variable automated statistical linear model

Item	Least square estimate	SE	*P*-value
K × Cu	1,889.00	529.30	<0.001
Mg	135.15	34.49	<0.001
Ca	4.69	0.80	<0.001
NDF × Zn	4.69	1.27	<0.001
P^2	3.84	0.42	<0.001
NDF × Na	1.75	0.22	<0.001
CP	0.68	0.10	<0.001
NDF × CP	- 0.03	<0.01	<0.001
Ca × P	- 2.49	0.46	<0.001
K	- 14.45	3.24	<0.001
Na × P	- 25.53	3.91	<0.001
Mg^2	- 107.72	29.79	<0.001
Zn	-108.60	29.70	<0.001
Cu^2	- 318,000.00	93,608.00	<0.001

DISCUSSION

Two linear models were used in the present study to describe the relationships between excreta composition (CP, NDF, and minerals) and excreta moisture. An automated objective statistical approach including all single variables, squared variables, and relevant interactions was compared with a biologically inspired method based on our current knowledge from the literature. As such, the biological approach may be perceived as subjective. In the automated model procedure an iterative process was used to derive an optimal solution, all combinations of variables were recursively fitted, and the fit statistics were recorded. For the models containing 14 variables the comparison of the automated

statistical model with the biologically compiled model showed that the 2 models had 9 components in common. These were Zn, Ca, CP, and K content and the interactions between Ca × P, K × Cu, NDF × Na, NDF × CP, and NDF × Zn content. Consequently, 5 components were unique for each of the 2 models. The automated statistical model contained 8 variables that were originally included in the biological model with 6 variables remaining, which potentially could mathematically improve the biological model. Of these, one (the interaction of K × Cu) was selected for inclusion in the biological model to improve its performance, resulting in an improvement in R^2 from 0.581 to 0.583. No other changes indicated by the automated statistical model improved the explained fraction of data variance and inclusion of some even reduced the R^2.

For the models containing 10 variables the differences between the automated and biological models were large, with only 4 variables in common to both models. The final biological model included K and CP content and not the interactions of NDF × Na and NFD × K content. Replacing K and CP by these 2 interactions as indicated by the automated statistical model improved the model R^2 from 0.530 to 0.542. Other changes reduced the explained fraction of the variance by the model. Adding additional parameters to the model can potentially improve the variance explained by the model. However, this can lead to an overly optimistic model (the problem of overfitting of the model), where excreta components are added without significantly improving model performance. This approach does not necessarily yield an improved predictive model and may result in models that cannot be replicated with other datasets (Babyak, 2004). An approach was chosen to define the number of components based on change of R^2. Subsequent to the 14 original excreta predictor variables, the addition of a single or pair of predictor variables resulted in only a minor (< 0.01) improvement in the model R^2. This approach was supported by the change in R^2 values in the automated model, which showed peaks at 10 and 14 predictor components in the model with little subsequent improvement (Fig. 1).

The biological model contained both fixed and random effect variables whereas a limitation of the automated model macro (Tilea et al., 2012) confined variables to include solely fixed effects. This resulted in different outcomes for the 2 models. The full model predictor variable set used by the automated model macro included 48 predictor variables and explained less variation with an R^2 of 0.468 compared with the mixed model with 14 variables and an R^2 of 0.583. When using Kramer's (2005) method to calculate R^2, error variance associated with random effects is partitioned from the true error reducing the true

error value and increasing the R^2 value. These findings indicate that the inclusion of random effects (mixed model) resulted in an improved model compared with including fixed effects only. Furthermore, the biological model was composed based on biological relevance and statistical contribution whereas the automated model was assembled using only a statistical algorithm.

The models indicate that mineral output makes an important contribution to excreta moisture output. Included in the model were Na, P, Ca, and K content, where increased excretion of Na, P, and Ca resulted in greater moisture excretion whereas increased K excretion resulted in lower excreta moisture. The greater excreta moisture or litter wetness scores with elevated dietary Na has been found by various authors (Murakami et al., 1997; Smith et al., 2000b; Oviedo-Rondón et al., 2001; Borges et al., 2003b; Jankowski et al., 2011). This finding is related to a linear increase in water intake with increasing dietary Na levels (Smith et al., 2000b; Ahmad et al., 2009) or increased osmolality of the digesta, which prevents water reabsorption in the hindgut. In feces of pigs, minerals have been reported to be the main osmotic particles (Etheridge et al., 1984). Excess in dietary Na levels increased the absorption of this mineral and hence increased osmolality of the blood, resulting in an increased Na excretion by the kidneys (Vena et al., 1990). Dietary salt content affects the osmotic pressure of the blood when absorbed, which is a thirst regulating mechanism (Borges et al., 2003a). As a result, birds will consume more water and if the digesta has a greater osmotic value, this will result in additional water output via the excreta (Vena et al., 1990; Smith et al., 2000b).

Increased dietary P content resulted in a linear increase in water intake and subsequent greater excreta moisture in laying hens (Smith et al., 2000b). The results of P in our model were in agreement with these findings. Decreasing dietary P levels reduces excretion of P, indicating the ability to manipulate excreta P content via diet (Ziaei et al., 2008). Increased dietary K content in laying hens (Smith et al., 2000b) or reduced K retention in broilers (Ziaei et al., 2007) resulted in greater excreta moisture. In contrast, a greater excreta K level reduced excreta moisture level in our model even though the range of dietary K levels tested was small compared with the range tested by Smith et al. (2000b). However, these authors did not measure the output of the minerals in the excreta, which may be a factor explaining the difference. Additionally, differences in strain or age of the laying hens may have caused variability in the response. Indeed, an increased litter moisture with increasing broiler age was demonstrated by Eichner et al. (2007) and was related to an increased water to feed ratio with

age (Ziaei et al., 2008). Also, the model presented here included age as a factor affecting excreta moisture level, indicating an age dependent effect on excreta moisture. However, the effect was contradictory to Eichner et al. (2007), as in the present study increasing age reduced excreta moisture. The age data in our analysis may have been confounded by the effect of barn, where all samples collected at 17 d of age were collected from barn 2. As barn was included as a random effect variable in the models, this interaction effect was not testable.

Crude protein was included in the model and an increased nitrogen excretion was related to greater excreta moisture content. This is in line with results reported by Ferguson et al. (1998a) and Namroud et al. (2008). Elevated dietary protein content (overformulation of diets) or an imbalanced amino acid profile has been identified as a cause of wet litter (Collett, 2006; Namroud et al., 2009). The excess protein supply is catabolized and used as an energy source and the nitrogen is excreted as uric acid by birds. Increasing the dietary CP level from 17 to 23% has been shown to result in an increased amount of uric acid, nitrogen, and moisture excreted on a excreta DM basis (Namroud et al., 2009). Unabsorbed dietary nutrients (e.g., protein and carbohydrates) at the end of the small intestine are a potential substrate for the microbiota in the distal GIT (distal ileum and ceca) (Apajalahti et al., 2004). Fermentation of protein produces metabolites that are harmful for the host and increase digesta pH (Apajalahti, 2005). Increased protein content of the digesta in the hindgut can, therefore, affect microbial composition in the hindgut and affect water reabsorption. However, in a previous experiment no relationship of microbial composition and excreta moisture content was observed (van der Hoeven-Hangoor et al., 2013c).

Besides known associations between excreta content of nutrients and moisture in broilers, hitherto undocumented associations were also observed. These included responses to excreta Zn content and several interactions between nutrient contents listed as follows: Ca × P, K × Cu, NDF × CP, NDF × Na, and NDF × Zn. The majority of experiments have measured the effect of increasing dietary levels of a single mineral on excreta moisture content or the ratio between Na, K, and Cl, expressed as the dietary electrolyte balance (**dEB**) (Ravindran et al., 2008). The dEB expresses the ability of Na^+ and K^+ to neutralize hydroxyl groups (OH^-) and Cl^- to neutralize hydrogen ions (H^+). Increasing dEB increases excreta moisture levels (Ahmad et al., 2009), because of increased Na (Ravindran et al., 2008) or K intake (Ahmad and Sarwar, 2006). An increase of dietary Ca or P levels did not show consistent effects on excreta moisture (Ferguson et al., 1998a; Smith et al., 2000b; Ziaei et al.,

2008). This may be related to the lower electrolytic capacity of divalent ions compared with monovalent ions or because of the presence of a relationship between Ca and P, as shown here. A relationship between Ca and P is known in relation to bone deposition, where both minerals are required for bone mineralization and a shortage of one mineral may result in losses of the other (Létourneau-Montminy et al., 2010). Fecal P content has been shown to rapidly increase when optimum tibia ash content is reached (Waldroup et al., 2000). A negative correlation between Na and K was reported in humans with diarrhea (fecal moisture above 80%) (Nishimuta et al., 2006), with lower K levels when Na increased, resulting in a greater fecal moisture content. Additionally, in laying hens, P increased excreta moisture especially at high dietary Na levels (Smith et al., 2000b). In contrast, our model did not identify a direct interaction between Na and K or P. The apparently contradictory results for some interactions could be a result of anomaly in the different datasets used to study this relationship. Several interactive effects between minerals (e.g., Na and Zn) and NDF on excreta moisture were observed. These may be explained by ion-exchange properties of insoluble dietary fibers, where binding is dependent on the concentration of the minerals (Laszlo, 1987). Nondetergent fiber measures lignin, hemicellulose, and cellulose as major components (van Soest et al., 1991) and was used as an indicator of insoluble fiber content. Available data are limited to in vitro studies and additional experiments are required to further explore these interactions.

Trace mineral (e.g., Mn, Zn, and Cu) concentrations in the excreta are closely related to dietary intake although their level does not affect excreta moisture (Zhong et al., 2007). In contrast, feeding increasing dietary Mg levels increased excreta moisture output in broilers (van der Hoeven-Hangoor et al., 2013b), which was not confirmed in the present experiment. In the models, increasing Zn levels reduced excreta moisture. Zinc is well known as a diarrhea treatment in piglets (Fairbrother et al., 2005) and humans (Patel et al., 2011). The proposed mechanism is through inhibition of Cl secretion into the lumen and enhancement of Na absorption, thereby preventing water excretion because of osmotic differences between serum and digesta (Hoque et al., 2009). Additionally, effects of Zn on microbial composition throughout the intestinal tract in newly weaned piglets have been observed (Højberg et al., 2005), which may affect fermentation of undigested nutrients and subsequent water reabsorption. Indeed, ceca and mid-colon DM content was found to be increased (approximately 10 and 20%, respectively) when higher Zn levels were fed to the piglets (Højberg et al., 2005). Typically, trace minerals are added to the diet using a premix, without

taking into account the trace mineral content of the macro ingredients, thereby supplying levels usually greater than specified. More in-depth evaluation of the effect of Zn on excreta moisture in broilers seems warranted.

Several nutrients such as excreta fat, soluble fiber content, starch, uric acid, and Cl content are missing in the database and were not taken into account by the model. They may have a relationship with excreta moisture, although literature of the effects of these nutrients on excreta quality is limited. Except for the effect of soluble nonstarch polysaccharides content, which has been observed to increase digesta viscosity (Ouhida et al., 2000; Jiménez-Moreno et al., 2013a) and concomitant increased water intake by the birds (Langhout et al., 2000), resulting in increased excreta and litter moisture content. Being part of the dEB calculation, Cl may be important to add to the database. However, no relationship between Cl and litter wetness score (Murakami et al., 1997; Murakami et al., 2001) or excreta moisture (Oviedo-Rondón et al., 2001) have been observed. Furthermore, diet characteristics (e.g., diet form, particle size, and pelleting temperature) could affect water intake or digesta composition in the hindgut and thereby change water reabsorption due to osmotic value.

Biological and automated statistical approaches were used to create 4 models to identify excreta nutrients that are associated with moisture content in broiler excreta. Increased excretion of Na, P, and Ca was shown to be associated with greater moisture excretion whereas increased K excretion was associated with lower excreta moisture. Several nutrients that have been identified previously were confirmed to affect excreta moisture content. Furthermore, hitherto undocumented excreta nutrient content (Zn) and interactions between excreta nutrient contents (NDF × CP, NDF × K, and Ca × P) that contribute to excreta moisture level were identified warranting further investigation to elaborate how they contribute to excreta moisture levels. As excreta levels of most nutrients can be manipulated by adjusting dietary levels, dietary formulation can be informed with the findings of this analysis permitting altered nutrient levels in the excreta.

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Chapter 4

Effect of different magnesium sources on digesta and excreta moisture content and production performance in broiler chickens

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ABSTRACT

Reducing litter moisture is an effective measure to reduce the incidence of footpad dermatitis. Dietary mineral levels affect intestinal conditions with regard to osmolarity and water reabsorption. Magnesium is often used as a laxative, preventing reabsorption of water from the digesta, and as a consequence, more moisture in the excreta. The objective of the current experiment was to evaluate Mg in broiler diets as a model for reduced intestinal water reabsorption. Effects of Mg source (magnesium sulfate, magnesium oxide, and magnesium chloride), each at 3 levels (0.255, 1.02, and 2.04 $g \cdot kg^{-1}$ diet), were studied. Measured effects were digesta moisture levels throughout the gastrointestinal tract and the moisture level of the excreta. The 10 treatments were randomly assigned to cages within 6 blocks, resulting in 6 replicates per treatments with 18 birds per replicate. Adding Mg to the diet of broilers linearly increased the excreta moisture content, following the pattern $MgCl > MgSO_4 = MgO$. This rejects the hypothesis that MgO and MgCl are less laxative sources compared with $MgSO_4$. The Mg sources most likely changed the water reabsorption in the distal gastrointestinal tract, as confirmed by the increased digesta moisture percentage in the ceca and colon. Increasing dietary $MgSO_4$ linearly reduced BW gain and feed intake, though absolute differences were minor. The results of this experiment show that Mg addition in the diet may be used as a model to study wet litter caused by reduced intestinal water reabsorption.

Key words: broiler, magnesium, excreta moisture, free water

INTRODUCTION

Broilers are usually housed on litter, which is primarily composed of bedding material (e.g., wood shavings) that is continuously mixed with feed, feathers, and excreta throughout the growing period (Stephenson et al., 1990; Torok et al., 2009). In practice, litter moisture concentrations range between 15 and 45% (Groot Koerkamp, 1994; Hayes et al., 2000) and vary according to season (Meluzzi et al., 2008). Litter is able to absorb large amounts of moisture, depending on the type of material used (Mayne et al., 2007; Abd El-Wahab et al., 2011). When the amount of water added exceeds the amount of water removed by evaporation, "wet litter" can occur. Wet litter is a multifactorial problem. The main factors affecting wet litter prevalence are management and housing of birds (Weaver Jr. and Meijerhof, 1991; Mitran et al., 2008), disease control, dietary factors (Francesch and Brufau, 2004), and gut health (Montagne et al., 2003). Preventing wet litter is a major focus in today's poultry industry to prevent production losses and maintain animal health and welfare. Multiple authors (Mayne et al., 2007; Wu and Hocking, 2011; Youssef et al., 2011b) suggest that reducing litter moisture is an effective measure to reduce the incidence of footpad dermatitis (**FPD**). The critical litter moisture content for FPD development is around 35% (Abd El-Wahab, 2011) with FPD linearly increasing at higher litter moisture concentrations (Meluzzi et al., 2008; Wu and Hocking, 2011).

Dietary mineral levels affect the osmolarity of the intestinal content and water reabsorption and as such, addition of high levels of minerals in the diet can therefore lead to diarrhea. However, the contribution of individual minerals to digesta osmolarity is diverse (Etheridge et al., 1984). Magnesium is frequently used as a laxative in humans (Vu et al., 2000; Schiller, 2001) and also as an osmotic agent in animal diarrhea models where supplementation with 2 $g \cdot kg^{-1}$ magnesium sulfate ($MgSO_4$) consistently induces diarrhea in rats (Galvez et al., 1993; Uddin et al., 2005; Antonisamy et al., 2009; Ikarashi et al., 2011). In humans, $MgSO_4$ decreases the small intestine transit time of chyme, reducing the time for nutrient and water reabsorption (Vu et al., 2000). In broilers, Lee and Britton (1983) also found a dose related reduction in gut digesta transit time with increasing dietary MgO levels. The $MgSO_4$ is not readily absorbed in the intestine and changes the osmotic pressure in the lumen as well as the expression of aquaporin 3 in rats. Due to the reduced water transport by aquaporin 3, reabsorption of water from the digesta is prevented, and as a consequence, more moisture is excreted (Ikarashi et al., 2011).

Magnesium, an essential cation in the diet of most animals (Lee and Britton, 1980), is involved in many cellular functions and is a cofactor in all major metabolic pathways (Saris et al., 2000; Liu et al., 2007a). Dietary addition of Mg under practical farming conditions is unusual, even though a recent study showed enhanced hepatic catalase activity after Mg supplementation (Liu et al., 2007a). This enhancement decreases lipid and muscle tissue peroxidation and subsequently can improve meat quality (Guo et al., 2003). However, high levels of Mg negatively affect bone calcification (Atteh and Leeson, 1983) and induce diarrhea (Lee and Britton, 1987) in broilers. Absorption of Mg takes place mainly in the duodenum, ileum, and colon (Guenter and Sell, 1973; Saris et al., 2000). In chickens, 72% of the dietary $MgSO_4$ intake is absorbed during gut passage; however, availability is limited to essentially zero due to a large proportion of Mg that is excreted via the kidneys (Guenter and Sell, 1973). Bioavailability of Mg depends on the source of Mg as has been shown for organic and inorganic Mg sources (Liu et al., 2007a) and on the dissociation of the ions in the intestine. Both Mg and SO_4 are poorly used ions (Schiller, 2001). Therefore, it may be more appropriate to choose a different Mg source to include in broiler diets. Magnesium oxide (MgO) and magnesium chloride (MgCl) are less toxic compared with $MgSO_4$ (Durlach et al., 2005) and thought to be less laxative (NRC, 2005). Besides the nutritional effects of Mg, it may also serve as a wet litter model in broilers, increasing excreta moisture by reduced water reabsorption in contrast to increased water consumption. This model could facilitate the development of dietary or management intervention studies to reduce wet litter problems in practice.

The objective of the current experiment was to evaluate the effect of different Mg sources on digesta moisture levels in different segments of the gastrointestinal tract (**GIT**), excreta moisture content, and production performance of broiler chickens. Furthermore, each Mg source was included at different levels to assess if digesta and excreta moisture are affected in a dose-related manner. Finally, the possibilities to use dietary Mg addition as a practical model to induce increased excreta moisture by reduced water reabsorption in the GIT were evaluated.

MATERIALS AND METHODS

Birds and Housing

The experiment was performed in a broiler unit consisting of 2 rooms of 30 cages each. Across both rooms, cages were divided over 6 blocks of 10 cages with 3 blocks in each room. One thousand eighty Ross 308 male 1-d-old chicks, derived from 33-wk-old broiler breeders, were purchased from a commercial hatchery (Lunteren, the Netherlands) and randomly allocated to 60 cages. At 7 d of age, all birds were individually weighed and based on weight classes, and birds weighing between 131 and 200 g were assigned to 1 of 10 cages of each of the 6 blocks in such a way that each treatment was represented in each block and in each room. Each cage consisted of 18 birds and had similar total weights per cage within block. Birds outside the weight range were not used. Average initial weight of the birds was 164.8 ± 4.4 g. The cages (100 x 110 cm) had a raised wire floor on top of which a rubber plate was placed, which was covered with a 2-cm layer of wood shavings. Each cage was equipped with 2 adjustable cup drinkers and a feeder that was positioned inside the cage for the first 14 d. From d 15 onward, feed was supplied via a feeder trough in front of the cage. Both feed and water were provided ad libitum throughout the study. Continuous artificial lighting was maintained for 23 h/d throughout the experiment. Temperature, relative humidity, and ventilation were computer controlled with the temperature gradually decreasing by 2.5°C per week, from 34°C on the day of arrival (1-d-old chicks) to a final temperature of 20.5°C at the end of the experiment (d 36). Room temperature was recorded continuously using data loggers, and relative humidity was set at 50% throughout the experiment. The birds were spray-vaccinated against Newcastle disease (Poulvac NDW-vaccine) at 13 d of age. The experimental methods were approved by the Ethical Committee of the Animal Science Group of Wageningen University and Research Center, Lelystad, the Netherlands.

Experimental Design

Three different Mg sources ($MgSO_4$, MgCl, and MgO) at 3 different Mg levels (0.255, 1.02, and 2.04 $g \cdot kg^{-1}$ diet) were tested against a control diet without any added Mg, resulting in a total of 10 treatments. Inclusion levels were chosen based on levels previously tested by Guo *et al.* (2003) and Liu *et al.* (2007a). In these studies the tested levels had little effects on

Table 1. Ingredient and nutritional composition of the experimental diets

Item	Starter diet, 7-14 d	Grower diet, 15-36 d
Ingredient composition, g·kg^{-1}		
Corn	419.1	447.0
Wheat	100.0	100.0
Soybean meal (>48% CP)	305.3	272.0
Toasted soybeans	50.0	50.0
Animal fat	22.0	40.2
Soya oil	22.0	13.4
Premix starter[1]	10.0	-
Premix grower[2]	-	10.0
Limestone	15.5	13.7
Monocalcium phosphate	13.5	11.3
NaCl	2.32	2.28
Sodiumbicarbonate	1.70	1.57
Celite[3]	10.0	10.0
DL-Methionine	2.40	2.15
L-Lysine HCL	1.17	1.28
L-Threonine	0.09	0.15
Variable composition	25.0	25.0
Calculated chemical composition, g·kg^{-1}		
Crude ash	85	77
AMEn[4] (poultry), kcal·kg^{-1}	2952	3054
AMEn (broiler), kcal·kg^{-1}	2750	2850
AFD Lys[5]	10.20	9.70
AFD Met	5.01	4.72
AFD Met + Cys	7.65	7.28

AFD Thr	6.43	6.11
AFD Trp	2.12	1.97
AFD Ile	7.85	7.15
AFD Arg	12.65	11.54
AFD Val	8.16	7.76
Linoleic acid	29.3	26.1
dEB[6], meq	240	234
Analyzed composition, $g \cdot kg^{-1}$		
CP	215	202
Crude fat	77	88
Crude fiber	31	32
DM	896	900
Ca	10.6	9.6
P	7.0	6.2
Na	1.7	1.7
K	9.2	8.8
Mg	2.0	1.9
Cl	2.2	2.4

[1]Contributed per kilogram of diet: riboflavin, 4.5 mg; niacinamide, 40 mg; D-pantothenic acid, 9 mg; choline chloride, 500 mg; cyanocobalamin, 20 µg; vitamin E (DL-α-tocopheryl acetate), 30 mg; menadione, 2.3 mg; vitamin A (retinyl acetate), 12,500 IU; cholecalciferol, 5,000 IU; biotin, 0.1 mg; folic acid, 0.5 mg; $FeSO_4 \cdot H_2O$, 147 mg; MnO_2, 100 mg; $CuSO_4 \cdot 5H_2O$, 40 mg; $ZnSO_4 \cdot H_2O$, 143 mg; Na_2SeO_3 0.5 mg; KI, 2 mg; antioxidant (oxytrap PXN), 125 mg.

[2]Contributed per kilogram of diet: riboflavin, 4.5 mg; niacinamide, 30 mg; D-pantothenic acid, 8 mg; choline chloride, 400 mg; cyanocobalamin, 20 µg; vitamin E (DL-α-tocopheryl acetate), 20 mg; menadione, 2.3 mg; vitamin A (retinyl acetate), 10,000 IU; cholecalciferol, 2,000 IU; biotin, 50 µg; folic acid, 0.5 mg; $FeSO_4 \cdot H_2O$, 147 mg; MnO_2, 100 mg; $CuSO_4 \cdot 5H_2O$, 40 mg; $ZnSO_4 \cdot H_2O$, 143 mg; Na_2SeO_3, 0.5 mg; KI, 1.9 mg; antioxidant (oxytrap PXN), 125 mg.

[3]Acid insoluble ash.

[4]Calculated according to CVB (2006).

[5]AFD = apparent fecal digestible, calculated according to CVB (2006).

[6]dEB = dietary electrolyte balance.

broiler performance. The 10 treatments were randomly assigned to cages within 6 blocks, resulting in 6 replicates per treatment with 18 birds per replicate. The experimental treatment started at 7 d of age. All birds received the same standard starter diet during the pre-experimental period.

Experimental Diets

All diets were formulated to meet the nutrient requirements of broilers (CVB, 2006). The starter and grower diets contained 2,952 and 3,054 kcal AME·kg^{-1} and 10.20 and 9.70 g·kg^{-1} apparent fecal digestible lysine, respectively. In advance of diet formulation, batches of wheat, corn, and soybean meal were reserved and analyzed for CP (Combustion, ISO 16634). Near Infrared reflectance spectroscopy (**NIRS**) analysis (ISO 12099) was applied to estimate crude ash, crude fat, crude fiber, and DM content.

For the preparation of the starter and grower diets, a basal diet (97.5%, Table 1) was produced and divided into 10 equal batches. In the control starter and grower diets, 1.25% Diamol and 1.25% cellulose were included as filler. The Mg sources were added at the desired levels via inclusion of $MgSO_4 \cdot H_2O$ (20.2% Mg), $MgCl \cdot 6H_2O$ (12.0% Mg), or MgO (60.3% Mg) based on their Mg content, exchanging on an equal weight basis with Diamol and cellulose (50% each). The exact inclusion of the different Mg sources and the analyzed Mg and Cl content of the produced diets are given in Table 2. Starter diets were pelleted at 2.5 mm and grower diets at 3.0 mm. After production, all diets were analyzed for N (Combustion, ISO 16634), crude fat, crude fiber, and DM (NIRS, ISO 12099), Ca, P, Na, K, and Mg (ICP-AES, ISO 27085:2009) content. Additionally, the control and MgCl diets were also analyzed for Cl content (Ionchromatography; Smee et al., 1978).

Data Collection

Bird weights were recorded per pen at the start of the experiment (d 7) and for individual birds at 14, 21, 28, and 36 d of age. In addition, feed consumption for each pen was recorded on the same days as the birds were weighed. Based on BW gain and feed consumption, feed conversion ratio (**FCR**, kg feed consumed/kg weight gain) was calculated.

Table 2. Composition of the variable components to the experimental diets, consisting of Diamol, cellulose and Mg source according to treatment and the analyzed Mg and Cl content of the diets after production

	Sulfate level, g·kg^{-1}				Oxide level, g·kg^{-1}			Chloride level, g·kg^{-1}		
Item	0.000[1]	0.255	1.020	2.040	0.255	1.020	2.040	0.255	1.020	2.040
Ingredient composition, g·kg^{-1}										
Diamol	12.50	11.85	9.95	7.35	12.25	11.50	10.50	11.45	8.25	3.95
Cellulose	12.50	11.85	9.95	7.35	12.25	11.50	10.50	11.45	8.25	3.95
Magnesium sulfate[1]	-	1.30	5.10	10.30	-	-	-	-	-	-
Magnesium oxide[2]	-	-	-	-	0.50	2.00	4.00	-	-	-
Magnesium chloride[3]	-	-	-	-	-	-	-	2.10	8.50	17.10
Analyzed composition, g·kg^{-1}										
Mg (starter)	2.0	2.2	3.0	3.6	2.3	2.8	3.6	2.2	2.9	3.9
Mg (grower)	1.9	2.3	2.7	3.8	2.3	2.8	4.2	2.2	2.9	3.9
Cl (starter)	2.2	-	-	-	-	-	-	4.3	3.2	6.4
Cl (grower)	2.4	-	-	-	-	-	-	3.0	5.3	8.4

[1]Control.
[2]$MgSO_4.H_2O$ (monohydrate): 20.2% Mg. K+S Kali GmbH, Kassel, Germany.
[3]MgO: 60.3% Mg. Timab, Dinard, France.
[4]$MgCl.6H_2O$ (hexahydrate): 12.0% Mg. Macco Oraniques Bruntál, Czech Republic.

At 14 and 28 d of age, excreta were collected per cage in one room (3 replicates per treatment) and at 36 d all cages were sampled (6 replicates per treatment). Excreta collection at 14 and 28 d was achieved using an excreta collection box, whereas at 36 d, litter and the rubber plate were removed from the cages and metal plates were placed underneath the cages from which excreta was collected. All excreta were collected per cage with feathers and feed particles carefully removed. Excreta samples were homogenized and a representative subsample was taken for DM analysis by NIRS (Smith et al., 2001; Hangoor et al., 2009). The amount of free water in each excreta sample was determined using the method Etheridge et al. (1984) with some modifications. An amount of excreta (22.0 ± 0.4 g) was accurately weighed into a preweighed plastic centrifuge tube and centrifuged at 2,230 x *g* for 24 min. Afterwards, the supernatant was manually removed using a pipette before the tube was reweighed. The amount of water removed by centrifugation (free water) was calculated by the difference and expressed as a percentage of the original excreta weight.

At d 36 of age, 4 broilers per cage with weights close to the mean weight of the cage were selected for sample collection. Birds were killed by cervical dislocation, and the GIT was removed and divided into 7 parts (duodenum, proximal jejunum, distal jejunum, proximal ileum, distal ileum, ceca, and colon) from which digesta was collected by gently squeezing (without the use of water) for DM analysis by freeze drying. The jejunum was taken to be between the end of the duodenal loop to Meckel's diverticulum and the ileum from Meckel's diverticulum to the ileocecal junction. Both were divided in 2 parts with equal length.

Statistical Analysis

For comparison of the different treatments, all data were subjected to mixed models analysis using the PROC MIXED procedure in SAS (version 9.2, 2008, SAS Institute Inc., Cary, NC) according to the following statistical model:

$$Y_{ij} = \mu + \tau_i + B_j + \varepsilon_{ij}$$

where Y_{ij} = specific trait measured for each experimental unit, μ = overall mean for the specific trait, τ_i = fixed effect of treatment (i = I, II, III, ..., X), B_j = random block effect (j = 1, 2, 3, ..., 6), and ε_{ij} = residual error term. Preplanned contrasts were used to determine significant relationships for 1) linear effect of $MgSO_4$ addition, 2) quadratic effect of $MgSO_4$ addition, 3) interactive effect of linear addition of $MgSO_4$ by linear addition of MgO, 4)

interactive effect of linear addition of $MgSO_4$ by linear addition of MgCl, and 5) interactive effect of linear addition of MgO by linear addition of MgCl.

RESULTS

Digesta DM

Increasing the dietary $MgSO_4$ concentration linearly increased digesta moisture in the ceca ($P < 0.0001$) and the colon ($P < 0.0001$; Table 3). No statistically significant quadratic effects were found (data not shown). Differences in the linear increase in digesta moisture to added Mg were found between the 3 Mg sources. In the proximal ileum, MgCl reduced digesta moisture with increasing dietary Mg level compared with $MgSO_4$ ($P = 0.0380$) and MgO ($P = 0.020$). Additionally, in the ceca, MgCl increased digesta moisture more than MgO ($P = 0.049$).

Excreta Moisture and Free Water

Excreta moisture increased linearly with increasing dietary $MgSO_4$ levels both at 14 ($P = 0.012$) and 36 ($P < 0.0001$) d of age (see Table 4). No statistically quadratic effects were found (data not shown). At 14 d of age, birds fed additional MgCl showed a stronger increase in excreta moisture compared with $MgSO_4$ ($P = 0.033$) and at 36 d of age birds fed additional MgCl had a stronger increase in excreta moisture compared with birds fed MgO ($P = 0.009$).

Free water in excreta increased linearly with increasing dietary $MgSO_4$ levels at 36 d of age ($P < 0.0001$, Table 4). No quadratic effects were found (data not shown). The linear response differed for MgO and $MgSO_4$ compared with MgCl. At 36 d of age, MgCl showed a stronger increase in excreta-free water compared with both $MgSO_4$ ($P = 0.009$) and MgO ($P = 0.0006$).

Table 3. The effect of Mg sources and inclusion level on digesta moisture (%) in different gastrointestinal tract segments of broiler chickens[1]

		Mg source and inclusion level, g·kg^{-1}											Contrast			
	Control	Sulfate (SO_4)			Oxide (O)			Chloride (Cl)			Pooled	Model	Linear	Linear Mg effect		
Item	Diet[2]	0.255	1.020	2.040	0.255	1.020	2.040	0.255	1.020	2.040	SEM	*P*-value	$MgSO_4$	SO_4 vs. O	SO_4 vs. Cl	O vs. Cl
Digesta moisture, %																
Duodenum	79.9	81.4	81.8	81.0	83.2	81.2	80.9	80.2	81.1	81.4	0.92	0.460	NS	NS	NS	*
Jejunum																
Proximal	71.0	67.9	61.1	67.9	69.2	75.1	67.0	61.5	66.4	67.0	4.21	0.419	NS	NS	NS	NS
Distal	61.8	58.5	53.7	56.4	53.8	59.3	58.8	55.3	55.6	57.2	4.94	0.970	NS	NS	NS	NS
Ileum																
Proximal	72.5	76.1	76.3	77.4	75.5	71.3	77.6	76.9	77.5	68.4	2.57	0.112	NS	NS	**	**
Distal	71.1	74.8	77.7	76.3	77.4	78.3	76.5	76.4	77.7	78.6	1.76	0.126	*	NS	NS	NS
Ceca	77.8	81.4	84.4	88.9	81.3	82.4	86.1	80.3	84.1	89.1	0.99	<0.0001	***	NS	NS	**
Colon	77.7	80.3	81.7	83.4	80.6	79.9	81.9	80.2	80.8	81.8	0.86	0.002	***	NS	NS	NS

[1]n = 6 replicates for all treatments.
[2]No added Mg.
NS, $P > 0.10$; *$0.10 < P \leq 0.05$; ** $0.05 < P \leq 0.01$; *** $P < 0.01$.

Table 4. The effect of Mg sources and inclusion level on excreta moisture level (%) and excreta free water (%) of broiler chickens

		Mg source and inclusion level, g·kg^{-1}											Contrasts			
	Control	Sulfate (SO_4)			Oxide (O)			Chloride (Cl)			Pooled	Model	Linear	Linear Mg effect		
Item	Diet[1]	0.255	1.020	2.040	0.255	1.020	2.040	0.255	1.020	2.040	SEM	*P*-value	$MgSO_4$	SO_4 vs. O	SO_4 vs. Cl	O vs. Cl
Excreta, 14 d																
n	3	3	3	3	3	3	3	3	3	3	-	-	-	-	-	-
Moisture	69.2	69.1	69.9	72.0	68.2	70.4	72.0	68.3	72.3	74.9	0.99	0.0002	**	NS	**	*
Free water	1.8	1.2	2.8	4.4	2.2	5.9	7.2	0.2	8.3	14.2	2.54	0.003	NS	NS	**	**

Excreta, 36 d																
n	4	6	5	4	5	5	6	5	6	6	-	-	-	-	-	-
Moisture	68.2	69.3	71.5	73.2	70.2	70.4	72.6	71.2	73.4	76.3	0.75	<0.0001	***	NS	NS	***
Free water	1.3	1.7	6.2	14.0	2.3	4.5	11.8	3.5	12.8	23.5	1.78	<0.0001	***	NS	***	***

[1] No added Mg.
NS, $P > 0.10$; *$0.10 < P \leq 0.05$; **$0.05 < P \leq 0.01$; *** $P < 0.01$.

Table 5. The effect of Mg sources and inclusion level on BW gain, feed intake and feed conversion ratio of broiler chickens[1]

		Mg source and inclusion level, g·kg^{-1}											Contrast			
	Control	Sulfate (SO_4)			Oxide (O)			Chloride (Cl)			Pooled	Model	Linear	Linear Mg effect		
Item	Diet[2]	0.255	1.020	2.040	0.255	1.020	2.040	0.255	1.020	2.040	SEM	*P*-value	$MgSO_4$	SO_4 vs. O	SO_4 vs. Cl	O vs. Cl
BW gain, g																
7 to 14 d	288	292	284	287	290	270	271	283	287	292	5.94	0.013	NS	NS	NS	***
14 to 36 d	2032	2064	2038	1987	2005	2028	2002	2015	2017	2004	23.2	0.311	**	*	NS	NS
7 to 36 d	2320	2355	2323	2274	2295	2297	2272	2299	2304	2297	25.5	0.235	**	NS	*	NS
Feed intake, g																
7 to 14 d	411	418	403	407	414	393	394	407	412	417	6.05	0.017	NS	NS	*	**
14 to 36 d	3479	3484	3422	3395	3437	3456	3419	3446	3461	3465	34.3	0.485	**	NS	*	NS
7 to 36 d	3890	3903	3825	3802	3851	3849	3813	3854	3873	3882	38.1	0.384	**	NS	*	NS
Feed conversion ratio																
7 to 14 d	1.428	1.437	1.419	1.417	1.430	1.458	1.456	1.438	1.434	1.428	0.018	0.400	NS	NS	NS	NS
14 to 36 d	1.713	1.688	1.679	1.708	1.714	1.705	1.708	1.711	1.717	1.729	0.011	0.091	NS	NS	NS	NS
7 to 36 d	1.677	1.657	1.647	1.672	1.678	1.675	1.678	1.677	1.681	1.691	0.010	0.057	NS	NS	NS	NS

[1] n = 6 replicates for all treatments.
[2] No added Mg.
NS, $P > 0.10$; *$0.10 < P \leq 0.05$; **$0.05 < P \leq 0.01$; ***$P < 0.01$.

Performance

Performance data are presented in Table 5. During the grower period (14 to 36 d) and total period (7 to 36 d), feeding increasing levels of $MgSO_4$ linearly reduced BW gain (P = 0.030 and P = 0.029, respectively). Similarly, feed intake linearly reduced after feeding increased $MgSO_4$ levels in the grower and total period (P = 0.015 and P = 0.016, respectively). In contrast, FCR responded in a quadratic manner to $MgSO_4$ in the grower and in the total period (P = 0.015 and P = 0.012, respectively), with the best FCR at 1.020 $g \cdot kg^{-1}$ Mg supplied by $MgSO_4$.

The linear response of feed intake and BW gain from 7 to 14 d of age to additional MgO differed from MgCl (P = 0.013 and P = 0.008, respectively). Adding MgO reduced BW gain and feed intake, whereas these parameters were slightly increased by the addition of MgCl.

DISCUSSION

The Mg sources tested in current study all clearly increased excreta moisture content. Excreta moisture increased on average with 5.5% due to any dietary Mg addition compared with the control diet. This finding is in agreement with findings by other authors (Lee and Britton, 1983;1987). It is interesting that the increase in excreta moisture output in our study was dependent on the source of dietary Mg. Compared with the control diet, adding MgO increased excreta moisture by 4.2%, $MgSO_4$ by 4.5%, and MgCl by 7.9%. The increase in excreta moisture followed a linear dose-response pattern. In broilers (Lee and Britton, 1983; 1987) and humans (Vu et al., 2000), a dose-related reduction in gut passage time with Mg supplementation was observed. Lee and Britton (1987) found a dietary addition of 0.3% MgO to be sufficient to induce diarrhea in broilers. In present study, a dietary Mg addition of 0.255% already increased excreta moisture, although no diarrhea was observed. Only at the highest Mg inclusion levels (2.04%) diarrhea seems to occur, with excreta moisture values around 75%.

Magnesium is classified as an poorly absorbable ion (Schiller, 2001), with a retention coefficient of 0.24 in 14-d-old broilers (Thomas and Ravindran, 2010) and between 0.15 and 0.18 in 21-d-old broilers (Ravindran et al., 2006). The presence of nonabsorbed minerals can be attributed to the osmolarity of the digesta (Etheridge et al., 1984), and the laxative effect of

Mg has been suggested to be due to osmotic effect of Mg (Schiller, 2001; Xing and Soffer, 2001). However, Lee and Britton (1987) did not find a difference in total osmolarity in the upper or low intestine after increasing Mg from 0.15% to 0.80%. These authors suggested that Mg-induced diarrhea was due to neural and endocrine effects rather than solely by osmotic effect.

In the present experiment, the slope of the increase in excreta moisture was different for the 3 Mg sources tested, suggesting that the Mg as oxide or the 2 counterions affected the response to the dietary Mg level differently. Magnesium oxide resulted in the least steep slope (1.56), followed by $MgSO_4$ (1.91) and MgCl (2.97), as shown in Figure 1. It is expected that solubility affects the bioavailability of Mg and this solubility affects the dissociation of the Mg source into ions and subsequently the level of Mg^{2+} present in the digesta at the lower intestine. Differences in the bioavailability of Mg sources were already apparent between organic and inorganic Mg sources, where organic sources induced a higher increase in serum Mg compared with inorganic Mg sources (Liu et al., 2007a). In humans, MgCl showed to have a higher bioavailability than MgO (Firoz and Graber, 2001). Additionally, Durlach et al. (2005) noted higher absorption levels of Mg from MgCl compared with $MgSO_4$. Magnesium plays a role in the opening, closing, or blocking of different ionic channels, affecting ion fluxes through membranes. Previous effect depends on the hydration state of the molecule, different between Mg salts (Guiet-Bara et al., 2007). In a aqueous solution, $MgSO_4$ is in a higher state of hydration with 7 water molecules compared with 6 water molecules in $MgCl_2$ (Durlach et al., 2005). A higher bioavailability and dissociation of the Mg sources into ions for MgCl compared with MgO and $MgSO_4$ results in an increased absorption of Mg and Cl. However, excess of both ions will be excreted via the kidneys (Saris et al., 2000) and can reach the colon and ceca via antiperistaltic movements, thereby affecting the osmotic value of the chyme and preventing water reabsorption.

Digesta moisture level changed throughout the digestive tract (Table 3). Our data in broilers showed increased moisture content of the digesta in the distal ileum, ceca, and colon after dietary addition of Mg. This finding may coincide with the primary function of the colon and ceca, which is absorption of fluids and ions (Holtug et al., 1996; Laverty et al., 2006). Urine excreted into the cloaca can due to antiperistalsis enter the colon and ceca, but not the small intestine (Clench and Mathias, 1995). The order of water reabsorption decreases from ceca > colon > coprodeum (Thomas, 1982; Clench and Mathias, 1995). Removing the ceca reduced DM digestibility (Raharjo and Farrell, 1984; Son et al., 2000) and increased the water

intake of birds (McNab, 1973). However, after some days, compensation of water reabsorption by other intestinal segments occurs. Through reflux into the colon and ceca, the Mg concentration in these segments could increase considerably with increasing dietary inclusion. The increased Mg concentration in the ceca and colon would subsequently reduce water reabsorption as shown by the linear increase in digesta moisture levels in those segments in the present study.

Dietary mineral level and dietary electrolyte balance (**dEB**) play an important role in excreta moisture (Collett, 2006). Essential minerals in the dEB calculation are monovalent electrolytes Na, K, and Cl ($dEB = Na^+ + K^+ - Cl^-$). Increasing Na alone or in combination with Cl in the diet (Murakami et al., 2001; Oviedo-Rondón et al., 2001; Jankowski et al., 2011) or drinking water (Watkins et al., 2005) increased excreta moisture content. The dietary salt content affects the blood osmotic pressure, which is a thirst-regulating mechanism (Borges et al., 2003a). As a result, broilers will consume more water and consequently this will result in extra water output via the excreta (Vena et al., 1990; Smith et al., 2000b). However, excreta moisture in broilers was not affected by Cl alone (Pesti et al., 1999; Murakami et al., 2001; Oviedo-Rondón et al., 2001). Chloride is considered the main regulator of the osmotic gradient responsible for water movement into the intestine in humans (Murek et al., 2010). In the present study, the digesta in the ceca and colon as well as the excreta of the birds fed the MgCl contained more water. Magnesium ($^{2+}$) and sulfate ($^{2-}$) have also been included in dEB calculation, although their importance is less compared with the basic 3 minerals in the calculation (Mongin, 1981). The reason for the latter is related to the lower absorption of divalent ions from the intestine into the blood and the fact that these minerals are usually oversupplied in the diet and therefore not limiting (Ahmad and Sarwar, 2006).

The binding of the water in the excreta of the birds was affected by Mg source, as shown by the differences in percentage excreta-free water (Table 4; Figure 1). Compared with the control diet, adding MgO in the diet increased free water on average 4.9 times, $MgSO_4$ 5.8 times, and MgCl 10.5 times. The increase in excreta-free water content with the MgCl treatment was significantly different from the MgO- and $MgSO_4$-supplemented diets. The increase in free water content in response to Mg addition for all Mg sources was linear. The increase of excreta-free water in the MgCl treatment was stronger (slope 11.2) compared with the other 2 Mg sources tested (slopes 6.9 and 5.4 for $MgSO_4$ and MgO, respectively). The binding of water affects the availability of water for the microbiota present in the litter and may therefore contribute to production of ammonia and its release from the litter (Groot

Koerkamp et al., 1998; Nahm, 2003). Therefore, not only reduction in excreta moisture content, but more importantly reduction of excreta-free water should be reached to reduce ammonia release from the litter.

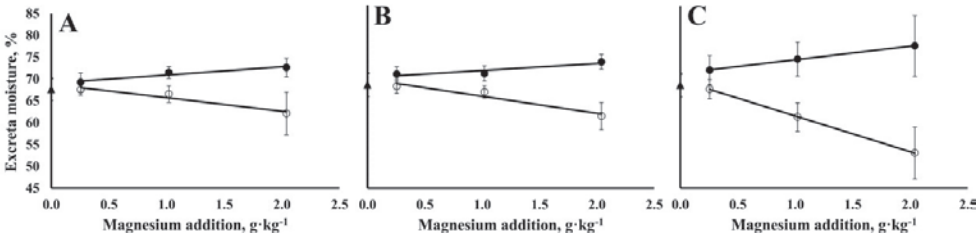

Figure 1. Percentage of excreta total (●) and bound moisture (○) at 36 d of age from birds without dietary Mg addition (▲) or fed increasing $MgSO_4$ (**A**), MgO (**B**), and MgCl (**C**) levels.

In agreement with studies by Guo et al. (2003) and Liu et al. (2007a), the tested levels of Mg had a limited effect on broiler performance, although BW gain and feed intake were linearly reduced by increasing $MgSO_4$. Magnesium is an important cation in the diet of most animals (Leeson and Summers, 2001), and it is essential for normal nerve conduction, muscle function, and bone mineralization (NRC, 2005). Additionally, Mg is an important cofactor in major metabolic pathways in the body (NRC, 2005) such as oxidative phosphorylation (Vitale et al., 1957) and the transfer of P between adenosine triphosphate, adenosine diphosphate, and adenosine monophosphate leading to adenosine triphosphate formation (Leeson and Summers, 2001). However, it has been suggested not to initiate Mg supplementation at a very young age (Atteh and Leeson, 1983; Gaal et al., 2004), especially because feeding excessive Mg (e.g., 1.4% $MgCO_3$) may lead to skeletal abnormalities (Lee and Britton, 1980).

In summary, adding Mg to the diet of broilers increased the digesta and excreta moisture content in a linear manner. This increase was highest for MgCl, followed by $MgSO_4$ and MgO, and rejects the hypothesis that MgO and MgCl are less laxative Mg sources compared with $MgSO_4$. The Mg sources most likely changed water reabsorption in the distal GIT, as confirmed by an increase in digesta moisture percentage in those segments. The later finding confirms the hypothesis that dietary Mg addition can be used as a model to study wet litter caused by reduced intestinal water reabsorption. Limited differences in excreta moisture content were found between the MgO and $MgSO_4$. Increasing dietary $MgSO_4$ significantly reduced BW gain and feed intake, although absolute differences were small. No significant

effects of MgCl and MgO addition were found compared with the dietary $MgSO_4$ addition. Therefore, it seems possible to use Mg as a wet litter model, where highest excreta moisture increase was obtained with MgCl.

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Chapter 5

Evaluation of free water and water activity measurements as functional alternatives to total moisture content in broiler excreta and litter samples

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ABSTRACT

Litter moisture contents vary greatly between and within practical poultry barns. The current experiment was designed to measure the effects of 8 different dietary characteristics on litter and excreta moisture content. Additionally, free water content and water activity of the excreta and litter were evaluated as additional quality parameters. The dietary treatments consisted of nonstarch polysaccharide content (NSP; corn versus wheat), particle size of insoluble fiber (coarse versus fine grinded oat hulls), viscosity of a non-fermentable fiber (low- and high-viscosity carboxymethyl cellulose), inclusion of a clay mineral (sepiolite), and inclusion of a laxative electrolyte ($MgSO_4$). The 8 treatments were randomly assigned to cages within blocks, resulting in 12 replicates per treatments with 6 birds per replicate. The dietary treatments had limited effects on excreta or litter water activity, and indications that this measurement in high moistures samples is limited were observed. Increasing dietary NSP content by feeding a corn-based diet (low NSP) compared with feeding a wheat-based diet (high NSP) increased water intake, excreta moisture and free water, and litter moisture content. Adding insoluble fibers to the wheat-based diet reduced excreta and litter moisture content, as well as litter water activity. Fine grinding of the oat hulls diminished the effect on litter moisture and water activity. However, excreta moisture and free water content were similar when fed finely or coarsely ground oat hulls. The effects of changing viscosity and adding a clay mineral or laxative deviated from results observed in previous studies. Findings of the current experiment indicate a potential for excreta free water measurement as an additional parameter to asses excreta quality. The exact implication of this parameter warrants further investigation.

Key words: broiler, feed composition, litter moisture, excreta moisture, water activity

INTRODUCTION

Litter moisture content in poultry production can vary greatly, ranging from 15 to 45% (Groot Koerkamp, 1994; Hayes et al., 2000; Miles et al., 2011b). The main input of water to the litter is via excreta, which is high in moisture and N (Nahm, 2003) although other inputs such as water spillage also contribute. Organic N present in the litter can be transformed to aerial ammonia by bacteria and fungi (Carlile, 1984; Cook et al., 2011), where higher litter moisture levels can be accompanied by an increased ammonia production (Groot Koerkamp, 1994; Miles et al., 2011a;b;c). High levels of ammonia may impair the health of birds (Kristensen and Wathes, 2000). Water in litter is used for dissolution of solid urea and subsequent urea hydrolysis (Nahm, 2003). Additionally, microbes need water for growth (Brown, 1976). However, water in litter can be bound to solutes (protein, fibers, and electrolytes) or be present in a free form. The strength of the water binding is dependent on the type of bond (e.g., ionic, covalent, hydrogen, or enclosure in capillaries; Chaplin, 2003). As more functional alternatives to total moisture content, free water (van der Hoeven-Hangoor et al., 2013b) and water activity ($\mathbf{A_w}$; Payne et al., 2007) may be used to assess the state of water in excreta and litter. Free water is defined here as 'the fraction of water that can be removed from the solution by a specific centrifuging force'. Free water indicates the mechanical properties of water in a sample and includes the water entrapped in capillaries. Although, depending on the g-force applied during centrifuging, also some water that was bound by weak hydrogen bonds can be included. Therefore, the value of free water can vary depending on the centrifuging speed applied. Water activity is a measure for the thermodynamics of water in a sample, related to the escaping tendency of water (Agoda-Tandjawa et al., 2013). Water activity at a given temperature is, "*the ratio of equilibrium partial vapor pressure of water in the system to the equilibrium partial vapor pressure of pure liquid water at the same temperature*" (Reid, 2007). Water activity indicates the available water component (Opara et al., 1992) and correlates well with microbial growth (Scott, 1957). Therefore, A_w may be a better measure to assess excreta and litter quality than total moisture or free water content. Data on A_w in broiler litter and broiler excreta are mainly related to pathogen growth; e.g., reduced *Salmonella* growth was found at excreta A_w levels below approximately 0.85 (Himathongkham et al., 1999; Payne et al., 2007).

Food ingredients (e.g., protein, starch, and cellulose) are known to differ in A_w characteristics (Labuza and Altunakar, 2007), providing potential to manipulate poultry excreta and litter A_w by dietary ingredients. Additionally, there is a strong relationship

between moisture and A_w (Himathongkham et al., 1999), making ingredients that affect excreta moisture possible candidates to change excreta and litter A_w. Previous research in broilers showed that the water content of excreta can be changed by adding Mg as a laxative to the diet (van der Hoeven-Hangoor et al., 2013b). However, A_w was not determined in the latter study. In contrast, clay minerals are often used as antidiarrhoeaics in human medicine (Carretero and Pozo, 2010). Clay minerals have a high water sorption capacity (i.e., absorption plus adsorption) due to their large surface area and the presence of micropores and channels (Galan, 1996). Sepiolite has been shown to reduce digesta viscosity and improved excreta scores in broilers fed high nonstarch polysaccharide (**NSP**) diets (Ouhida et al., 2000) and may be a potential candidate to change litter free water and A_w values.

Soluble NSP increase excreta and litter moisture due to an increased digesta viscosity (Ouhida et al., 2000; Jiménez-Moreno et al., 2013a) and concomitant higher water intake by the birds (Langhout et al., 2000). However, it is uncertain how dietary NSP content and viscosity of the digesta affect excreta and litter A_w, as soluble NSP are potent water binders. On the other hand, insoluble NSP have a high water holding capacity (Chaplin, 2003). Furthermore, they increase gizzard activity and subsequent digesta passage rate (Hetland et al., 2004). The effects of insoluble NSP on digestion seems to depend on particle size. Coarse oat hulls increased digesta passage rate compared with finely ground hulls (Hetland and Svihus, 2001), although coarse particles reduced excreta moisture content (Amerah et al., 2009; Jiménez-Moreno et al., 2013b). A high gizzard activity as a result of oat hull inclusion improved digestibility of starch (Amerah et al., 2009), CP, and DM (Jiménez-Moreno et al., 2013a). It is hypothesized that as a consequence of a reduced nutrient load in the hindgut, osmolality and water reabsorption are affected as well.

The aim of the current study was to evaluate the effects of dietary characteristics, including NSP content (corn versus wheat), particle size of insoluble fiber (coarse versus fine grinded oat hulls), viscosity of a non-fermentable fiber (low and high-viscous carboxymethyl cellulose), inclusion of a clay mineral (sepiolite), and inclusion of a laxative ($MgSO_4$) on moisture content and measurements of free water content and A_w of broiler excreta and litter.

MATERIALS AND METHODS

Birds and Housing

The experiment was performed in a broiler unit consisting of one room with 96 cages, divided into 6 rows. Each row was split into 2 blocks, resulting in a total of 12 blocks with 8 cages each. Five hundred seventy-six, 1-d-old male Ross 308 chicks, derived from 40-wk-old broiler breeders, were purchased from a commercial hatchery (Lunteren, the Netherlands) and allocated to 8 dietary treatments across 96 cages so that each treatment was represented in each block. Each cage housed 6 birds with an initial individual chick weight of 41.0 ± 1.3 g.

The cages (50 x 50 cm) had a raised wire floor with a metal plate on top, covered with a 2-cm layer of wood shavings. Litter was prevented from spreading to adjacent cages by placing vertical plastic plates of 20 cm height between cages. Each cage was equipped with 2 adjustable nipple drinkers and a feeder that was positioned inside the cage for the first 3 d. From 3 d onward, feed was supplied via a feeder trough in front of the cage. Both feed and water were provided ad libitum throughout the study. Continuous artificial lighting was maintained for 23 h/d for the first 3 d of the experiment, 20 h/d between 4 and 7 d, and 18 h/d for the remainder of the experiment. Temperature, relative humidity, and ventilation were computer controlled with the temperature gradually decreasing by 0.5°C per day, from 33.0°C on the day of arrival (1-d-old chicks) to a final temperature of 26.1°C at the end of the experiment (14 d). Room temperature was recorded continuously using data loggers, and relative humidity was set at 50% throughout the experiment. The study was approved by the Ethical Committee of the Animal Science Group of Wageningen University and Research Center, Lelystad, the Netherlands.

Experimental Diets

The 8 diets were formulated to meet the nutrient requirements of broilers (CVB, 2006) and to contain similar contents of ME (2,750 AME·kg^{-1}) and apparent fecal digestible lysine, methionine+cystine, threonine, and valine. In advance of diet formulation, batches of wheat, corn, and soybean meal were reserved and analyzed for CP (Combustion, ISO 16634, Rapid N Cube, Elementar GmbH, Hanau, Germany), and Ca and P content (ICP-AES, ISO 27085:2009, Thermo Iris Intrepid II XSP Duo, Thermo Scientific Inc., Waltham, MA). Near-

infrared reflectance spectroscopy analysis (Bruker MPA, ISO 12099, Bruker Optik GmbH, Ettlingen, Germany) was used to estimate DM, crude fat, crude fiber, and crude ash content.

The first experimental diet (**CORN**) was based on 61.2% corn, which can be considered an easily digestible diet for broilers due to a low NSP content. The second experimental diet (**WHEAT**) contained 60.6% wheat to obtain a higher level of NSP. Dietary contents were adjusted with SBM, soya oil, mineral sources, and synthetic amino acids to ensure that the diets were isonutritious and isocaloric on an AME basis. Other diets had test products added to the WHEAT diet: whole and hammer-milled oat hulls (Dhuyvetter BvBa, Kruishoutem, Belgium) were included at 2.5% to create a coarse oat hull (**cOH**) and a fine oat hull diet (**fOH**). Particle size distribution for the coarse oat hulls was: >5.6 mm 0%; >2.8 mm 55%; >2.5 mm 1%; >2.0 mm 9%; >1.7 mm 11%; >1.4 mm 7%; >1.0 mm 10%; >0.5 mm 4% and >0 mm 3%; and for the fine oat hulls: >2.8 mm 0%; >2.5 mm 0%; >1.4 mm 1%; >0.8 mm 25%; >0.5 mm 25% and >0 mm 49%, as determined by dry sieving. Furthermore, 1% carboxymethylcellulose sodium salt (Sigma-Aldrich, St. Louis, MO) with a low viscosity (50-200 mPa·s, 4% in H_2O; **lCMC**) and with a high viscosity (1,500-3,000 mPa·s, 1% in H_2O; **hCMC**) were added. Carboxymethylcellulose inclusion levels were as reported by Smits et al. (1997) and contain around 80 g $Na \cdot kg^{-1}$. Dietary Na level was not corrected in the lCMC and hCMC diet, as it is uncertain how much of the Na from CMC is available for absorption in the GIT. Finally, 1% sepiolite ($Mg_2H_2Si_3O_9 \cdot xH_2O$; Sigma-Aldrich, St. Louis, MO), was added as an water adsorbent clay mineral (**SEP**) and based on previous results (van der Hoeven-Hangoor et al., 2013b), 1.03% magnesium sulfate ($MgSO_4 \cdot H_2O$, 20.2% Mg; **$MgSO_4$**) was added as a laxative. It was decided not to use an inert filler, as these ingredients are similar to clay minerals, one of the test components. Therefore, test products were added by exchanging wheat on an equal weight basis, without any further correction.

The ingredient composition of the experimental corn and wheat diets is presented in Table 1. Diets were pelleted with steam addition (approximately 80°C) at 2.5 mm. After production, all diets were analyzed for CP (Combustion, ISO 16634, Rapid N Cube, Elementar GmbH, Hanau, Germany), crude fiber (AOCS Ba 6a-05, Ankom A 200, Ankom Technology, Macedon, NY), DM (Gavimetry, ISO 6496, Memmert UNB 500, Memmert GmbH, Schwabach, Germany), and Ca and P content (ICP-AES, ISO 27085:2009. Thermo Iris Intrepid II XSP Duo, Thermo Scientific Inc., Waltham, MA). Near-infrared reflectance spectroscopy analysis (Bruker MPA, ISO 12099, Bruker Optik GmbH, Ettlingen, Germany)

was applied to estimate crude fat content. Chemical composition of the experimental diets is provided in Table 2.

Table 1. Ingredient and nutritional composition of the experimental diets

Item	Corn diet	Wheat diet
Ingredient composition, $g \cdot kg^{-1}$		
Corn	612.1	-
Wheat	-	606.1
Soybean meal (>48% CP)	321.9	294.2
Soya oil	16.8	50.4
Premix starter[1]	10.0	10.0
Limestone	16.7	17.2
Monocalcium phosphate	14.0	14.0
Sodiumbicarbonate	3.02	2.61
NaCl	1.80	1.95
DL-Methionine	2.13	1.88
L-Lysine HCL	1.36	1.33
L-Threonine	0.09	0.28
Calculated chemical composition, $g \cdot kg^{-1}$		
CP	205	213
Crude fat	45	64
Crude fiber	27	24
DM	878	869
Crude ash	61	61
AME_n (poultry), $kcal \cdot kg^{-1}$, [2]	2907	2980
AME_n (broiler), $kcal \cdot kg^{-1}$	2750	2750
AFD Lys[3]	10.20	10.20
AFD Met	4.83	4.52
AFD Met + Cys	7.45	7.45
AFD Thr	6.43	6.43
AFD Trp	2.07	2.33
AFD Ile	7.51	7.55
AFD Arg	12.11	12.08
AFD Val	8.16	8.16
Linoleic acid	21.73	31.01
Ca	9.68	9.68
P	6.43	6.44
Na	1.60	1.60
K	9.27	9.12
Cl	1.80	1.80
Dietary electrolyte balance, mEq	256	252

[1]Contributed per kg diet: riboflavin, 4.5 mg; niacinamide, 40 mg; D-pantothenic acid, 9 mg; choline chloride, 500 mg; cyanocobalamin, 20 µg; vitamin E (DL-α-tocopheryl acetate), 30 mg; menadione, 2.3 mg; vitamin A (retinyl-acetate), 12,500 IU; cholecalciferol, 5,000 IU; biotin, 0.1 mg; folic acid, 0.5 mg; $FeSO_4 \cdot H_2O$, 147 mg; MnO_2, 100 mg; $CuSO_4 \cdot 5H_2O$, 40 mg; $ZnSO_4 \cdot H_2O$, 143 mg; Na_2SeO_3 0.5 mg; KI, 2 mg; antioxidant (oxytrap PXN), 125 mg.
[2]Calculated according to CVB (2006).
[3]AFD = apparent fecal digestible, calculated according to CVB (2006).

Table 2. Chemical composition of the experimental diets after production

Item	Corn	Wheat	cOH[1]	fOH[2]	lCMC[3]	hCMC[4]	SEP[5]	$MgSO_4$[6]
Analyzed composition, g·kg^{-1}								
CP	216	227	227	222	220	223	222	226
Crude fat	46	65	64	64	63	63	63	63
Crude fiber	20	22	29	26	22	21	22	22
DM	882	865	865	868	867	867	867	864
Ca	10.0	10.0	9.9	10.5	10.3	10.1	10.6	10.2
P	7.0	6.9	7.1	7.5	7.5	6.8	7.5	7.3

[1]Coarse oat hulls. Dhuyvetter BvBa, Kruishoutem, Belgium. Particle size distribution: >5.6 mm 0%; >2.8 mm 55%; >2.5 mm 1%; >2.0 mm 9%; >1.7 mm 11%; >1.4 mm 7%; >1.0 mm 10%; >0.5 mm 4% and >0 mm 3%.
[2]Milled oat hulls. Dhuyvetter BvBa, Kruishoutem, Belgium. Particle size distribution: >2.8 mm 0%; >2.5 mm 0%; >1.4 mm 1%; >0.8 mm 25%; >0.5 mm 25% and >0 mm 49%.
[3]1% carboxymethylcellulose sodium salt with a low viscosity: 50-200 mPa·s, 4% in H_2O. Sigma-Aldrich, St. Louis, MO.
[4]1% carboxymethylcellulose sodium salt with a high viscosity: 1500-3000 mPa·s, 1% in H_2O. Sigma-Aldrich, St. Louis, MO.
[5]1% sepiolite: $Mg_2H_2Si_3O_9 \cdot H_2O$. Sigma-Aldrich, St. Louis, MO.
[6]1.03% magnesium sulfate $MgSO_4.H_2O$ (monohydrate): 20.2% Mg. K+S Kali GmbH, Kassel, Germany.

Data Collection

Bird weights were recorded per pen at the start of the experiment (d 0) and for individual birds at 3 and 14 d of age. In addition, feed consumption for each pen was recorded on the same days as the birds were weighed. Based on BW gain and feed consumption, feed conversion ratio (**FCR**; kg of feed consumed/kg of weight gain) was calculated. Water intake was measured per cage using load cells continuously recording water bucket weights throughout the experiment. Water intake data are reported as values during the total period.

At 15 d of age, a litter sample (approximately 100 g) was taken from a spot in the middle of each cage, removing the full depth of the litter layer. Subsequently, the sample was mixed and divided into 2 subsamples for analysis of moisture content and A_w. Free water measurements were not suitable for litter samples, probably due to the relatively low moisture fraction. To facilitate excreta sampling, the litter and the rubber plate were removed from all cages, leaving the birds on the wire floor from 15 d of age onward. In the morning of d 16, collection plates were placed underneath each cage and excreta (approximately 100 g) were collected with feathers and feed particles removed in 3 or 4 intervals of 2 h each, depending on the excreta production. Excreta were refrigerated (4°C) in between intervals, pooled per cage, weighed, and thoroughly mixed. Homogenized excreta were divided into 2 subsamples for analysis of moisture content and A_w. In addition, free water content was determined using the method described previously by van der Hoeven-Hangoor et al. (2013b).

On 17 d of age, 5 birds per cage were weighed before being killed by cervical dislocation. From each bird, the jejunum (from the end of the duodenum to Meckel's diverticulum) and colon (from the ileo-cecal junction to the beginning of the cloaca) digesta contents were gently expelled by hand, collected, and pooled per segment and per cage. Digesta samples were frozen at -20°C for later DM analysis. In accordance with Kocher et al. (2000), part of the jejunum digesta was subjected to centrifugation at 12,000 x *g* by a temperature of 4°C for 10 min, and the supernatants stored overnight at 4°C. Subsequently, viscosity was analyzed using a Brookfield DV-I+ viscometer (Brookfield Engineering Laboratories, Inc, Middleborough, MA) with a CP 40 cone at 25°C. Each sample was measured in triplicate. When shear-thinning (i.e., when viscosity decreased with increasing shear rates instead of staying the same) occurred, the sample was measured at 4 to 5 different RPM to calculate viscosity at 100 RPM. Colon digesta osmolality was measured in duplicate with an Advanced Model 3320 Micro-Osmometer (Advanced Instruments Inc., Norwood, MA). Additionally, from one bird of each cage, the pH of the digesta in the gizzard was determined by inserting a pH-electrode (CyberScan pH 11, Eutech Instruments Pte Ltd, Singapore) immediately after dissection of the gizzard.

Moisture and A_w analysis

To determine litter and excreta moisture content, samples were oven dried at 70°C for 16 h, subsequently ground and dried at 103°C for another 4 h. Jejunum and colon digesta were freeze dried to determine moisture content. For both oven and freeze drying the difference in weight before and after drying was expressed relative to total sample weight. Fresh litter and excreta A_w were measured with a HygroPalm HP23 Handheld Indicator (Rotronic AG, Bassersdorf, Switzerland). The device uses an algorithm provided by the manufacturer to accelerate the A_w measurement and to provide a result within approximately 5 min. As specified by the manufacturer, the A_w value measured with this method deviates less than 0.005 from the full equilibration measurement. For litter samples, a fixed volume (approximately 40 mL) and for excreta samples, a fixed weight (approximately 30 g) was placed in the sensor chamber.

Statistical Analysis

For comparison of means among the different treatments, all data were subjected to mixed model analysis using the PROC MIXED procedure in SAS (version 9.2, 2008, SAS Institute Inc., Cary, NC) according to the following general linear model:

$$Y_{ijk} = \mu + \tau_i + B_j + R_k + \varepsilon_{ijk}$$

where Y_{ijk} = specific response measured for each experimental unit, μ = overall mean for the specific response, τ_i = fixed effect of treatment (i = I, II, III, ..., VIII); B_j = random block effect (j = A, B, C, ..., F); R_k = random row effect (k = X or Y), and ε_{ijk} = residual error term. Pen was the experimental unit. The following preplanned contrasts were used to determine significant relationships for 1) corn vs. wheat, 2) wheat vs. coarse oat hulls, 3) fine vs. coarse oat hulls, 4) low vs. high viscous CMC, 5) wheat vs. sepiolite, and 6) wheat vs. $MgSO_4$. Specific a-priori identification of these contrasts negated the need to conduct exhaustive all-pairwise comparisons among treatment means, reducing exposure of the results to the associated rise in experiment-wise error rates.

Nineteen of the 48 litter A_w data points exceeded the theoretical limit of 1.0, possibly due to variation in precision of the sensor. Therefore, values for all data points were decreased by the difference between the highest measured value and 1.0 (i.e., 0.007). Distributions of the means and residuals were examined to assess normality and homogeneity of the data (Ott and Longnecker, 2001). Litter and excreta A_w data and jejunum viscosity data were found to be non-normally distributed. Water activity data were subsequently normalized using an arc-sin square root transformation and jejunum viscosity by a natural logarithm transformation. Following transformation, it was evident that the transformed data had an improved normality profile that was sufficiently normalized permitting further analysis (Shapiro and Wilk, 1965). For litter and excreta A_w sample temperature was added as a covariate to the model, as A_w is depending on the temperature at the time of measurement (Roos, 2007). For jejunum viscosity measuring day was added as a random variable to the model to account for variation created by measuring on 2 subsequent days.

RESULTS

Excreta and litter characteristics

Excreta moisture content was higher ($P = 0.044$) in birds fed the CORN diet compared with birds fed the WHEAT diet (82.9% vs. 80.9%, Table 3). Replacing 2.5% wheat with coarse oat hulls resulted in a lower ($P = 0.035$) excreta moisture content (78.8% vs. 80.9%). Feeding the lCMC diet resulted in a higher ($P = 0.049$) excreta moisture content compared with feeding the hCMC diet (83.1% vs. 81.1%). None of the other treatments showed a significant effect on excreta moisture content.

Table 3. Fifteen-day-old litter moisture content, and excreta moisture- and free water content of 16-d-old broiler chickens fed various diets[1]

	Excreta, %		Litter, %
Item	Moisture	Free water	Moisture
Diet			
Corn	82.9	30.8	56.9
Wheat	80.9	18.3	44.8
cOH[2]	78.8	16.2	38.8
fOH[3]	79.4	20.9	46.8
lCMC[4]	83.1	29.9	51.9
hCMC[5]	81.1	19.9	46.6
SEP[6]	79.9	16.8	46.0
$MgSO_4$[7]	81.1	25.8	47.3
Pooled SEM	0.75	2.15	2.42
Treatment *P*-value	0.0001	<0.0001	<0.0001
P-values for preplanned contrasts			
Corn vs. wheat	0.044	<0.0001	0.0004
Wheat vs. coarse OH	0.035	0.501	0.069
Fine vs. coarse OH	0.561	0.123	0.017
Low vs. high viscous CMC	0.049	0.001	0.114
Wheat vs. sepiolite	0.285	0.645	0.725
Wheat vs. $MgSO_4$	0.851	0.015	0.457

[1]n = 12 replicates for all treatments.
[2]Coarse oat hulls. Dhuyvetter BvBa, Kruishoutem, Belgium. Particle size distribution: >5.6 mm 0%; >2.8 mm 55%; >2.5 mm 1%; >2.0 mm 9%; >1.7 mm 11%; >1.4 mm 7%; >1.0 mm 10%; >0.5 mm 4% and >0 mm 3%.
[3]Milled oat hulls. Dhuyvetter BvBa, Kruishoutem, Belgium. Particle size distribution: >2.8 mm 0%; >2.5 mm 0%; >1.4 mm 1%; >0.8 mm 25%; >0.5 mm 25% and >0 mm 49%.
[4]1% carboxymethylcellulose sodium salt with a low viscosity: 50-200 mPa·s, 4% in H_2O. Sigma-Aldrich, St. Louis, MO.
[5]1% carboxymethylcellulose sodium salt with a high viscosity: 1500-3000 mPa·s, 1% in H_2O. Sigma-Aldrich, St. Louis, MO.
[6]1% sepiolite: $Mg_2H_2Si_3O_9.H_2O$. Sigma-Aldrich, St. Louis, MO.
[7]1.03% magnesium sulfate $MgSO_4.H_2O$ (monohydrate): 20.2% Mg. K+S Kali GmbH, Kassel, Germany.

Excreta free water content of birds fed the CORN diet was higher ($P < 0.0001$) compared with birds fed the WHEAT diet (30.8% vs. 18.3%, Table 3). Feeding the lCMC diet resulted in a higher ($P = 0.001$) excreta free water content compared with feeding the hCMC diet (29.9% vs. 19.9%). Replacing 1.03% wheat by $MgSO_4$ resulted in a higher ($P = 0.015$) excreta free water content (25.8% vs. 18.3%). None of the other treatments showed a significant effect on excreta free water content. Excreta water activity was not affected by the dietary treatments tested (Table 4).

Litter moisture content was higher ($P = 0.0004$) in birds fed the CORN diet compared with birds fed the WHEAT diet (56.9% vs. 44.8%, Table 3). Replacing 2.5% wheat with coarse oat hulls numerically resulted in a lower ($P = 0.069$) litter moisture (38.8% vs. 44.8%). Fine grinding of the oat hulls resulted in a higher ($P = 0.017$) litter moisture content compared with coarse grinding of oat hulls (46.8% vs. 38.8%). None of the other treatments significantly affected litter moisture content.

Feeding birds the cOH diet resulted in a lower litter A_w ($P = 0.001$) compared with feeding the WHEAT diet (0.973 vs. 0.994, Table 4). Grinding of the oat hulls changed litter A_w, where feeding birds the cOH diet resulted in a lower ($P = 0.043$) litter A_w compared with feeding the fOH diet (0.973 vs. 0.987). None of the other treatments showed a significant effect on litter A_w.

Dissection results

Feeding the cOH diet increased relative gizzard weight by 0.46% ($P = 0.003$) compared with feeding the WHEAT diet and by 0.40% ($P = 0.009$) compared with the fOH diet (Table 5). None of the other treatments showed significant effects on relative gizzard weight. Gizzard pH was not affected by the experimental diets tested.

Digesta moisture content of the jejunum was lower ($P < 0.0001$) when the CORN diet was fed compared with feeding the WHEAT diet (81.4% vs. 83.3%, Table 5). Replacing wheat with 2.5% coarse oat hulls or with 1% sepiolite increased jejunum digesta moisture content by 1.2% ($P = 0.0002$) and by 0.7% ($P = 0.045$), respectively. Feeding the fOH diet resulted in a lower ($P < 0.0001$) jejunum digesta moisture content compared with feeding the cOH diet (83.2% vs. 84.5%).

Feeding birds the fOH diet resulted in a lower (P = 0.024) colon digesta moisture content compared with feeding the cOH diet (81.6%vs. 82.5%, Table 5). Birds fed the $MgSO_4$ diet had in a higher (P = 0.001) colon digesta moisture content compared with birds fed the WHEAT diet (83.5% vs. 82.0%). None of the other treatments showed significant effects on digesta moisture content of the jejunum or colon.

Table 4. Water activity 'A_w' in 15-d-old litter and excreta of 16-d-old broiler chickens fed various diets, including sample temperature as a covariable[1]

	Excreta water activity		Litter water activity	
Item	Mean[2]	95% CI[3]	Mean[2]	95% CI[3]
Diet				
Corn	0.979	0.963 – 0.990	0.994	0.988 – 0.998
Wheat	0.979	0.962 – 0.990	0.994	0.987 – 0.998
cOH[4]	0.979	0.962 – 0.990	0.973	0.960 – 0.983
fOH[5]	0.978	0.961 – 0.990	0.987	0.978 – 0.994
lCMC[6]	0.979	0.964 – 0.991	0.992	0.985 – 0.997
hCMC[7]	0.979	0.962 – 0.991	0.989	0.980 – 0.995
SEP[8]	0.980	0.963 – 0.991	0.993	0.985 – 0.998
$MgSO_4$[9]	0.980	0.964 – 0.991	0.994	0.988 – 0.999
Treatment *P*-value	0.417	-	0.014	-
Sample temperature *P*-value	0.097	-	0.006	-
P-values for preplanned contrasts				
Corn vs. wheat	0.697	-	0.872	-
Wheat vs. coarse OH	0.873	-	0.001	-
Fine vs. coarse OH	0.351	-	0.043	-
Low vs. high viscous CMC	0.534	-	0.521	-
Wheat vs. sepiolite	0.343	-	0.821	-
Wheat vs. $MgSO_4$	0.121	-	0.851	-

[1]n = 6 replicates for all treatments.
[2]Backtransformed arc-sin square root LSmeans.
[3]Backtransformed 95% confidence interval provided instead of SEM.
[4]Coarse oat hulls. Dhuyvetter BvBa, Kruishoutem, Belgium. Particle size distribution: >5.6 mm 0%; >2.8 mm 55%; >2.5 mm 1%; >2.0 mm 9%; >1.7 mm 11%; >1.4 mm 7%; >1.0 mm 10%; >0.5 mm 4% and >0 mm 3%.
[5]Milled oat hulls. Dhuyvetter BvBa, Kruishoutem, Belgium. Particle size distribution: >2.8 mm 0%; >2.5 mm 0%; >1.4 mm 1%; >0.8 mm 25%; >0.5 mm 25% and >0 mm 49%.
[6]1% carboxymethylcellulose sodium salt with a low viscosity: 50-200 mPa·s, 4% in H_2O. Sigma-Aldrich, St. Louis, MO.
[7]1% carboxymethylcellulose sodium salt with a high viscosity: 1500-3000 mPa·s, 1% in H_2O. Sigma-Aldrich, St. Louis, MO.
[8]1% sepiolite: $Mg_2H_2Si_3O_9 \cdot xH_2O$. Sigma-Aldrich, St. Louis, MO.
[9]1.03% magnesium sulfate $MgSO_4 \cdot H_2O$ (monohydrate): 20.2% Mg. K+S Kali GmbH, Kassel, Germany.

Table 5. Relative gizzard weight, gizzard content pH, jejunum and colon digesta moisture content and jejunum digesta viscosity of 17-d-old broiler chickens fed various diets

	Gizzard[1]		Digesta moisture, %[1]		Jejunum viscosity, mPa·s[2]	
Item	Relative weight, %	pH	Jejunum	Colon	Mean[3]	95% CI[4]
Diet						
Corn	1.95	3.00	81.4	82.0	1.89	1.54 – 2.30
Wheat	2.06	3.07	83.3	82.0	2.56	2.09 – 3.13
cOH[5]	2.52	2.89	84.5	82.5	2.69	2.21 – 3.27
fOH[6]	2.12	2.83	83.2	81.6	2.62	2.14 – 3.20
lCMC[7]	2.06	3.12	83.3	82.9	3.16	2.59 – 3.87
hCMC[8]	1.97	3.22	83.4	82.7	3.87	3.17 – 4.73
SEP[9]	1.95	3.19	83.9	82.2	2.44	1.99 – 2.98
$MgSO_4$[10]	1.91	3.00	83.7	83.5	2.42	1.98 – 2.96
Pooled SEM	0.11	0.10	0.27	0.37		
Treatment *P*-value	0.003	0.088	<0.0001	0.001	<0.0001	
P-values for preplanned contrasts						
Corn vs. wheat	0.465	0.614	<0.0001	0.939	<0.0001	
Wheat vs. coarse OH	0.003	0.216	0.0002	0.246	0.223	
Fine vs. coarse OH	0.009	0.688	<0.0001	0.024	0.511	
Low vs. high viscous CMC	0.582	0.489	0.635	0.748	<0.0001	
Wheat vs. sepiolite	0.457	0.391	0.045	0.653	0.227	
Wheat vs. $MgSO_4$	0.309	0.622	0.165	0.001	0.167	

[1]n = 12 replicates for all treatments. [2]n = 9 replicates for all treatments.
[3]Geometric LSmeans.
[4]Backtransformed 95% confidence interval provided instead of SEM.
[5]Coarse oat hulls. Dhuyvetter BvBa, Kruishoutem, Belgium. Particle size distribution: >5.6 mm 0%; >2.8 mm 55%; >2.5 mm 1%; >2.0 mm 9%; >1.7 mm 11%; >1.4 mm 7%; >1.0 mm 10%; >0.5 mm 4% and >0 mm 3%.
[6]Milled oat hulls. Dhuyvetter BvBa, Kruishoutem, Belgium. Particle size distribution: >2.8 mm 0%; >2.5 mm 0%; >1.4 mm 1%; >0.8 mm 25%; >0.5 mm 25% and >0 mm 49%.
[7]1% carboxymethylcellulose sodium salt with a low viscosity: 50-200 mPa·s, 4% in H_2O. Sigma-Aldrich, St. Louis, MO.
[8]1% carboxymethylcellulose sodium salt with a high viscosity: 1500-3000 mPa·s, 1% in H_2O. Sigma-Aldrich, St. Louis, MO.
[9]1% sepiolite: $Mg_2H_2Si_3O_9.H_2O$. Sigma-Aldrich, St. Louis, MO.
[10]1.03% magnesium sulfate $MgSO_4 \cdot H_2O$ (monohydrate): 20.2% Mg. K+S Kali GmbH, Kassel, Germany.

Jejunum digesta viscosity was higher ($P < 0.0001$) in birds fed the WHEAT diet compared with birds fed the CORN diet (2.56 and 1.89 mPa·s; Table 5). Feeding the hCMC diet resulted in a higher ($P < 0.0001$) jejunum digesta viscosity compared with feeding the lCMC diet (3.87 vs. 3.16 mPa·s). None of the other treatments showed significant effects on jejunum digesta viscosity.

Osmolality of the colon supernatant was not affected (P = 0.42) by feeding the WHEAT compared with feeding the CORN diet (Table 6). Feeding the $MgSO_4$ diet numerically resulted in a higher (P = 0.099) colon osmolality compared with feeding the WHEAT diet (397 vs. 372 mOsm·kg^{-1}).

Table 6. Colon osmolality of 17-d-old broiler chickens fed three experimental diets[1]

Item	Colon Osmolality, mOsm·kg^{-1}
Diet	
Corn	384
Wheat	372
$MgSO_4$[2]	397
Pooled SEM	12.6
Treatment *P*-value	0.246
P-values for preplanned contrasts	
Corn vs. Wheat	0.420
Wheat vs. $MgSO_4$	0.099

[1]n = 9 replicates for all treatments.
[2]1.03% magnesium sulfate $MgSO_4 \cdot H_2O$ (monohydrate): 20.2% Mg. K+S Kali GmbH, Kassel, Germany.

Production performance

Mortality rate was low with 0.35% (data not shown). Average BW at 14 d of age (442 g) was in line with Aviagen Ross 308 performance objectives (481 g). Production performance results are presented in Table 7.

Feeding the CORN diet increased ADG by 20.8% (P < 0.0001), ADFI by 18.7% (P < 0.0001), and reduced FCR by 1.7% (P = 0.0005) compared with feeding the WHEAT diet. Replacing part of the wheat with coarse oat hulls, sepiolite, or $MgSO_4$ had no effect on production performance. Feeding the cOH diet numerically deteriorated (P = 0.090) FCR by 0.9% compared with feeding the fOH diet. Birds fed the hCMC diet had a 7.1% higher (P = 0.010) ADG and 7.8% higher (P = 0.006) ADFI compared with birds fed the lCMC diet.

Average daily water intake increased (P = 0.0001) by 17.2% when feeding the CORN diet compared with feeding the WHEAT diet. None of the other treatments had significant effects on average daily water intake. The ratio of water to feed intake was not affected by the experimental diets tested.

Table 7. Body weight, average daily gain (ADG), average daily feed intake (ADFI), average daily water intake (ADWI), feed conversion ratio (FCR), and water to feed ratio (WFR) of broiler chickens fed various diets[1]

		0 to 14 d of age				
Item	BW 14d, g	ADG, g	ADFI, g	ADWI, g	FCR	WFR
Diet						
Corn	512	33.6	39.4	112.4	1.172	2.85
Wheat	431	27.8	33.2	95.9	1.193	2.89
cOH[2]	428	27.7	33.3	91.3	1.201	2.74
fOH[3]	440	28.5	33.9	97.5	1.191	2.88
lCMC[4]	415	26.8	31.7	97.3	1.185	3.07
hCMC[5]	442	28.7	34.2	99.8	1.191	2.92
SEP[6]	429	27.8	32.9	96.9	1.186	2.94
$MgSO_4$[7]	435	28.2	33.5	97.5	1.188	2.91
Pooled SEM	9.20	0.65	0.79	3.05	0.005	0.08
Treatment *P*-value	<0.0001	<0.0001	<0.0001	0.0004	0.0008	0.169
P-values for preplanned contrasts						
Corn vs. wheat	<0.0001	<0.0001	<0.0001	0.0001	0.0005	0.689
Wheat vs. coarse OH	0.820	0.826	0.947	0.263	0.172	0.149
Fine vs. coarse OH	0.280	0.263	0.456	0.134	0.070	0.180
Low vs. high viscous CMC	0.010	0.010	0.006	0.563	0.345	0.176
Wheat vs. sepiolite	0.896	0.945	0.752	0.819	0.202	0.605
Wheat vs. $MgSO_4$	0.648	0.602	0.745	0.708	0.337	0.834

[1]n = 12 replicates for all treatments.
[2]Coarse oat hulls. Dhuyvetter BvBa, Kruishoutem, Belgium. Particle size distribution: >5.6 mm 0%; >2.8 mm 55%; >2.5 mm 1%; >2.0 mm 9%; >1.7 mm 11%; >1.4 mm 7%; >1.0 mm 10%; >0.5 mm 4% and >0 mm 3%.
[3]Milled oat hulls. Dhuyvetter BvBa, Kruishoutem, Belgium. Particle size distribution: >2.8 mm 0%; >2.5 mm 0%; >1.4 mm 1%; >0.8 mm 25%; >0.5 mm 25% and >0 mm 49%.
[4]1% carboxymethylcellulose sodium salt with a low viscosity: 50-200 mPa·s, 4% in H_2O. Sigma-Aldrich, St. Louis, MO.
[5]1% carboxymethylcellulose sodium salt with a high viscosity: 1500-3000 mPa·s, 1% in H_2O. Sigma-Aldrich, St. Louis, MO.
[6]1% sepiolite: $Mg_2H_2Si_3O_9{\cdot}H_2O$. Sigma-Aldrich, St. Louis, MO.
[7]1.03% magnesium sulfate $MgSO_4{\cdot}H_2O$ (monohydrate): 20.2% Mg. K+S Kali GmbH, Kassel, Germany.

DISCUSSION

The dietary treatments, which consisted of various factors, i.e., modulating the NSP content (corn versus wheat), particle size of insoluble fiber (coarse versus fine grinded oat hulls), clay mineral (sepiolite), viscosity of a non-fermentable fiber (low and high-viscosity carboxymethyl cellulose), and a laxative electrolyte ($MgSO_4$), changed broiler excreta moisture, excreta free water, and litter moisture content. However, only small effects of the different treatments on excreta and litter A_w were observed. The latter is likely related to the high values of A_w measured which were all close to the maximum A_w value of 1.0. The moisture content of excreta measured in this experiment at 16 d of age was high (mean value of 80.9%) compared with a moisture content of 63.5% measured in 18 d-old broilers by Jiménez-Moreno et al. (2013a) and 70.6% measured in 14 d-old broilers by van der Hoeven-Hangoor et al. (2013b). Given the strong relationship between moisture and A_w (Himathongkham et al., 1999; Hayes et al., 2000), this probably resulted in the high excreta A_w values measured in this experiment. Also litter moisture content was high (mean value of 47.4%), which resembles values measured in caked litter areas (Sistani et al., 2003). Indeed, litter caking was observed at the end of the experiment. The high moisture content of the litter may also be related to the housing conditions. Six birds were housed per cage of 50 x 50 cm, with the feeder on one side of the cage and the drinking nipples on the other side. In practice, areas around drinkers are usually higher in moisture (Miles et al., 2013) and show more frequent caking of the litter (Miles et al., 2008) compared with other parts of the barn. The design of the experimental cages may, therefore, have interfered with the litter conditions. Still, Hayes et al. (2000) also found in 86 commercial poultry houses that 74.4% of the samples had an A_w greater than 0.90 and 72.1% of the samples had litter moisture contents above 30.0%. Only at litter moisture content below 26.0%, they found A_w to decrease rapidly. With the A_w values as observed in the current experiment (mean value of 0.99 for both litter and excreta), no effect on microbial activity was expected. For Salmonella, no growth inhibition has been observed at excreta A_w levels above 0.85, whereas A_w levels below 0.85 reduced growth (Himathongkham et al., 1999; Hayes et al., 2000; Payne et al., 2007). These findings indicate measuring A_w is less suitable for high moisture substrates like excreta and litter and had no discriminant ability in the current experimental setup.

To assess the effect of dietary NSP content and hence the digestibility of the diet, a low NSP corn-based diet was compared with a higher NSP wheat-based diet. In line with expectations, the birds fed the CORN diet had superior ADG, ADFI, and FCR compared with

birds fed the WHEAT diet. The lower production performance of the WHEAT diet has been related to a reduced digestibility of high viscous and high NSP content diets (Jørgensen et al., 1996; Knudsen, 1997; Jia et al., 2009). Interestingly, however, the main difference in litter moisture content was induced by feeding the better digestible, low NSP corn-based diet. Litter moisture increased by 12.1% in the CORN diet compared with the WHEAT diet. In line with litter moisture, excreta moisture increased by 2.0% and excreta free water content by 12.6% when the CORN diet was fed compared with the WHEAT diet. The results from the current experiment were opposite to expectations, as increasing soluble NSP content increased fecal moisture content in dogs (Twomey et al., 2003) and in broilers (Jiménez-Moreno et al., 2013a). It is hypothesized that as a result of the high feed intake of the birds fed the CORN diet, the transit time decreased, reducing the time for water reabsorption in the hindgut. The very high feed intake by the CORN fed birds resulted in a significantly higher excreta production (40.4 g/h for the CORN and 31.3 g/h for the WHEAT diet, data not shown), which in combination with a higher excreta moisture content, resulted in a higher litter moisture addition in the CORN treatment. In agreement with this hypothesis, a relationship between increased passage rate and higher feed intake (Almirall and Esteve-Garcia, 1994) and a strong relationship ($r = 0.83$) between transit time and fecal dry matter weight (Wiggins, 1984) have been previously observed. These findings suggest that besides digestibility, transit time may be important also for excreta moisture output.

The effect of adding insoluble fibers with different fiber particle sizes was evaluated by replacing 2.5% wheat in the WHEAT diet by coarse oat hulls. The cOH diet reduced litter A_w and numerically reduced litter moisture content by 6.0% compared with feeding the WHEAT diet. These results confirm the positive effect of coarse insoluble fibers on litter quality. Nonetheless, the absolute A_w value of 0.973 for the cOH diet is still too high to expect any effect on microbial growth in the litter. The effect of the cOH diet on litter moisture content and litter A_w disappeared when the oat hulls were finely grinded. These findings confirm the importance of larger particle sizes for dry litter. This is in line with findings by Amerah et al. (2009), who observed an improved litter score at 21 d of age when wood shavings were added to a control wheat diet, whereas finely ground cellulose had no effect on litter score. Additionally, feeding the cOH diet reduced excreta moisture by 2.1% compared with feeding the WHEAT diet, whereas excreta free water was not affected. The increased excreta DM with coarse oat hulls is in line with Jiménez-Moreno et al. (2013a), who

observed a numerical increase in 18 d of age excreta DM at an inclusion of 5.0 and 7.5% oat hulls, although not at 2.5%.

Conversely to litter moisture parameters, there was no effect of particle size of the oat hulls on excreta moisture or excreta free water content. Birds fed the fOH diet had similar excreta water content compared with the cOH diet. In line with results presented by Jiménez-Moreno et al. (2013a), feeding the cOH diet did not improve ADG or FCR compared with feeding the WHEAT diet. Relative gizzard weight of birds fed the cOH diet increased by 22.2% compared with the WHEAT diet and by 19.0% compared with the fOH diet, indicative of higher gizzard stimulation by increased particle size. Increased gizzard size was also found when feeding coarse particle diets (Amerah et al., 2009) and when including whole oats in the diet (Hetland et al., 2002). In the current experiment, gizzard pH was not affected by coarseness of the oat hulls. In line with previous observation, Hetland et al. (2002) found no effect of 1.25 or 3.0% whole oat inclusion on gizzard pH, although, Jiménez-Moreno et al. (2013b) observed a quadratic decline in gizzard pH with increasing oat hull inclusion from 2.5 to 7.5%. The lower inclusion level of oat hulls by Hetland et al. (2002) and in our experiment will have limited the effect on gizzard pH. The discrepancy in the results may indicate that a 2.5% oat hull inclusion is not enough to result in a consistent response in gizzard size, gizzard pH, and excreta moisture content to oat hulls. Additionally, the pelleting process may have reduced the particle size of the oat hulls, thereby limiting the effect of particle size on excreta parameters. Contradicting effects of coarse fiber inclusion in pelleted diets on production performance and digestibility have been described earlier in the scientific literature (Amerah et al., 2007). Litter moisture observations were more consistent and indicate a potential for coarse oat hulls to improve litter quality parameters.

Our aim was to change digesta viscosity by feeding a high or low viscous CMC and measure its effect on excreta and litter quality parameters. Higher digesta viscosity increases moisture content of the excreta (van der Klis et al., 1993a) Most likely this is related to reduced fecal digestibility of lipids, N (Smits et al., 1997), and minerals (K, P, Ca, and Mg) with increasing dietary CMC inclusion, coinciding with increased ileal digesta osmolality (van der Klis et al., 1993b). Increased moisture output has been related to mineral excretion previously, potentially related to increased digesta osmolality (van der Hoeven-Hangoor et al., 2013a). A small but significant increase from 3.16 to 3.87 mPa·s in jejunum viscosity was found between birds fed the low or high viscous CMC. These results are in line with findings by Ouhida et al. (2000), van der Klis et al. (1993a), and Smits et al. (1997). Although, the

increase from 1.2 to 8.1 mPa·s observed by van der Klis et al. (1993a) and 7.9 mPa·s to 17.4 mPa·s by Smits et al. (1997) were higher than the viscosities observed in the current experiment. The difference may be explained by the viscosity differences between CMC products tested, with 5,000-8,000 mPa·s for the hCMC product tested by van der Klis et al. (1993a), compared with 1,500-3,000 mPa·s for the product tested in the current experiment and by Smits et al. (1997). Changing digesta viscosity had no effect on litter moisture content or on litter A_w. Although feeding the lCMC diet increased excreta moisture content by 2.0% and excreta free water content by 10.0% compared with feeding the hCMC diet. The lower feed intake of the lCMC birds lowered excreta production compared with the hCMC birds (30.0 versus 32.0 g/h, data not shown), resulting in a lower total moisture addition to the litter compared with the hCMC diet. In contrast to the observations in the current experiment, Ouhida et al. (2000) found a higher percentage of birds with wet droppings (defined as birds with heavily adhered excreta) when birds were fed a diet with 1.0% dietary hCMC (87.5%) compared with a corn diet (29.2%). In line with Langhout and Schutte (1996), no differences in water intake or WFR were observed between the low and high viscous CMC in the present study. In contrast, van der Klis et al. (1993a) and Smits et al. (1997) measured a higher viscosity difference and an concomitant increase in both water intake and in WFR after adding viscous CMC. The small digesta viscosity difference between the lCMC and hCMC may have limited the physiological response in the current experiment, limiting the response in excreta and litter moisture content.

The lower excreta free water content of birds fed the hCMC is potentially related to water being trapped in a gel matrix in the more viscous digesta (Hetland et al., 2004). This limits release of the water after applying a centrifugal force. Similarly, lower excreta free water content was observed in birds fed the WHEAT diet compared with birds fed the CORN diet, which coincided with a higher jejunum digesta viscosity in the WHEAT fed birds. The increased jejunum digesta viscosity in the WHEAT diet is probably a result of a higher soluble NSP content of wheat (Knudsen, 1997; Kocher et al., 2000). This result is in line with findings by Ouhida et al. (2000). Additionally, Jia et al. (2009) observed a higher small intestine viscosity in birds fed a wheat diet compared with birds fed a corn diet (3.4 and 2.5 mPa·s). These results show that higher digesta viscosity reduces excreta free water content, although, the exact mechanism is not fully understood at this point. Our findings support the hypothesis that excreta free water determination provides a different quality measurement compared with total excreta water content.

Adding 1.0% sepiolite, a clay mineral with a high water absorbent capacity, to the wheat-based diet had no effect on litter or excreta moisture content and A_w, or excreta free water content. In contrasts, Ouhida et al. (2000) observed a reduced incidence of wet droppings, especially in high viscous diets, when adding 1.0 or 2.0% sepiolite to the diet. Their observation coincided with a reduced jejunum viscosity and a reduction in mean retention time in the gizzard at 1.0 or 2.0% inclusion of sepiolite compared with the control diet. In the current experiment, jejunum viscosity was not affected by adding sepiolite, and jejunum moisture content was 0.7% higher compared with the WHEAT diet. No clear explanation for this discrepancy is apparent. Also in contrast with previous observations (Lee and Britton, 1987; Ikarashi et al., 2011; van der Hoeven-Hangoor et al., 2013b) was the absence of an effect on excreta or litter moisture of adding a laxative ($MgSO_4$). The absence may have been related to the high average moisture content in the current experiment (80.9%), which was higher compared with that of our previous experiment (70.6%), and may have limited the effect of the laxative. However, in line with previous observation (van der Hoeven-Hangoor et al., 2013b), $MgSO_4$ increased excreta free water content by 7.6% and colon digesta moisture content by 1.4%. No change in water intake or WFR was observed, although a numerically higher colon digesta osmolality was measured in the $MgSO_4$ fed birds compared with the WHEAT fed birds. The latter results are most likely indicative for reduced water reabsorption in the hindgut. An inverse linear relationship between osmolality and net fluid absorption in the small intestine of pigs has been observed by Kiers et al. (2006). However, Ikarashi et al. (2011) observed that laxation induced by $MgSO_4$ is not solely associated to osmolality in rats, but also involves increased aquaporin 3 expression in the colon mucosal epithelial cells. Again, the difference in response between excreta moisture and excreta free water results is indicative of free water determination being a different quality measurement to assess excreta quality.

In summary, the results of the current experiment show limited effects of dietary NSP content, particle size of insoluble fiber, viscosity, clay mineral inclusion, or laxative capacity on excreta or litter A_w. Additionally, limitations of A_w measurements in high moistures samples are shown. Free water was affected by changing viscosity and by adding a laxative, supporting the hypothesis that free water determination provides a different quality measurement to assess excreta quality. Excreta and litter moisture content increased when a corn-based diet (low NSP) was fed compared with a wheat-based diet (high NSP) and indicate that transit time affects moisture intake and excretion. The effect of transit time on water

reabsorption warrants further investigation. Coarse oat hulls reduced excreta and litter moisture content and litter A_w compared with feeding a wheat-based diet. The importance of particle size was confirmed for litter moisture and A_w, as fine grinding of the oat hulls diminished this effect. However, excreta moisture and free water content was similar when finely or coarsely ground oat hulls were fed. The effects of changing digesta viscosity or adding a clay mineral or laxative deviated from results observed in previous studies.

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Chapter 6

General discussion

INTRODUCTION

In commercial broiler farms, birds are usually housed on litter, composed of a bedding material like wood shavings. Throughout the growing period, the litter is mixed with feed, feathers, and excreta (Cook et al., 2011). Typical litter moisture content ranges between 15 and 45% (Stephenson et al., 1990; Groot Koerkamp, 1994; Hayes et al., 2000; Miles et al., 2011b). Above a certain litter moisture content, "wet litter" can occur causing more ammonia to be produced and emitted into the air (Groot Koerkamp, 1994). This can negatively affect animal production and welfare (Kristensen and Wathes, 2000). Wet litter is described as a poor condition of litter in poultry barns, referring to a high litter moisture content and impaired bird performance. It has been reported in poultry already since 1950 (Wheeler and James, 1950). With more intensive poultry housing it is more often reported (Hermans et al., 2006). A consistent definition is lacking, although a comprehensive description is provided by Hermans et al. (2006) "*when material covering the floors of poultry houses reaches its saturation threshold and is unable to hold more moisture*".

The major difficulty of controlling the wet litter problem is that multiple causes exist and often more than one cause is involved in the manifestation of the problem. However, issues with wet litter can usually be connected to 2 major attributors: a too high input of moisture onto the litter or a too low output of moisture from the litter. Therefore, a problem as simple as a leaking nipple or a poor ventilation schedule can lead to a high moisture content of the litter. However, also causes related to nutritional disturbances which lead to intestinal infection, bacterial overgrowth, and coccidiosis (Tucker and Remus, 2001) can lead to higher moisture output by birds. Consequently, more moisture will be added to the litter. Management related issues leading to poor litter quality are well known (Weaver Jr. and Meijerhof, 1991; Tucker and Walker, 1992; Elwinger and Svensson, 1996; Hermans et al., 2006; Mitran et al., 2008). Also disease related issues leading to diarrhea are rather well studied, especially in humans (Gorbach et al., 1971; Ashkenazi and Pickering, 1989; Bovee-Oudenhoven et al., 2003; Freeman, 2005) and weaning piglets (Oli et al., 1998; Fairbrother et al., 2005; Castillo et al., 2008; Hu et al., 2012). Management and disease related factors were not in scope for this thesis.

Effects of nutrition on digestibility and excreta moisture content have been studied before. However, not much emphasis has been placed on the effect of dietary formulations on excreta quality itself. Often a fundamental approach was used, such as the effect of fibers on digesta viscosity (Wang et al., 1992; van der Klis et al., 1993b; Ouhida et al., 2000; Montagne

et al., 2003; Dikeman and Fahey Jr, 2006) and on gizzard development (Hetland and Svihus, 2001; Amerah et al., 2009; Jiménez-Moreno et al., 2013b). Also dietary electrolyte balance (Ahmad et al., 2009) including Na, K, and Cl (Smith et al., 2000b; Ahmad and Sarwar, 2006; Ravindran et al., 2008; Koreleski et al., 2011) and protein content have been studied, although the latter has been often confounded with the inclusion level of soybean meal (Elwinger and Svensson, 1996; Alleman and Leclercq, 1997; Francesch and Brufau, 2004; Namroud et al., 2009). In current thesis these factors are therefore not studied in itself.

In this thesis, the focus was to use practical dietary formulations and study how they relate to wet litter. It was aimed also to investigate different measures of excreta and litter water and evaluate the possible consequences for litter quality in apparently healthy broiler chickens by using different measures of excreta water. Throughout **Chapter 2 through 5** different nutritional aspects were studied for their effects on excreta moisture content. In the following sections an overview of the main findings regarding the effect of nutrition on excreta quality is provided. Additionally, different parameters to address excreta and litter quality have been evaluated and the main results will be discussed. Furthermore, at the end of this chapter the main conclusions and practical considerations based on the research presented in this thesis will be summarized.

NUTRITIONAL ASPECTS AFFECTING EXCRETA MOISTURE CONTENT

The gastro intestinal tract (**GIT**) of broilers is well adapted to absorb nutrients and water (Thomas, 1982; Goldstein and Skadhauge, 2000; Laverty and Skadhauge, 2008; McWhorter et al., 2009). Fresh droppings of broilers contain between 60-70% (Leeson et al., 1976) to 80% moisture (Henuk and Dingle, 2003). In this thesis the aim was to change excreta moisture output in apparently healthy broilers via nutrition. It is clear from the literature that dietary ingredient composition can affect digestibility of nutrients by the bird. As a result, differences in undigested nutrient in the hind gut digesta will appear. Subsequently, these undigested nutrients potentially act as osmotic particles or as substrate for microbial fermentation in the hindgut. Therefore, differences in nutrient digestibility could lead to excessive water loss via the excreta.

Table 1. Overview of the different dietary treatments tested throughout the different chapters, and how they affected digesta, excreta and litter characteristics

Diet	Chapter	Hypothesis	Jejunum viscosity	Digesta moisture		Colon Osmolality	Excreta, 14/15 d of age			Litter, 16 d of age		Excreta, 34/36 d of	
				Caeca	Colon		Moisture	A_w	Free water	Moisture	A_w	Moisture	Free water
Corn	1, 4	Good digestible, low NSP	=		=	=	↑	=	↑	↑	=	=	
Wheat	1, 4	Poor digestible, high NSP	↑		=	=	=	=	=	=	=	=	
Starch	1	Increase starch in hindgut										=	
Enzyme	1	Improved digestibility										=	
MCFA	1	Improved gut health										=	
$MgSO_4$	3, 4	Increase osmolality in hindgut	=	↑	↑	↑	↑ / =	=	↑ / =	=	=	↑	↑
MgO	3	Increase osmolality in hindgut		↑	↑		↑		↑			↑	↑
MgCl	3	Increase osmolality in hindgut		↑	↑		↑		↑			↑	↑
Coarse oat hulls	4	Improved digestibility	=		=		↓	=	=	↓	↓		
Fine oat hulls	4	Particle size fiber	=		↓		=	=	=	=	=		
Sepiolite	4	Water absorbent in hindgut	=		=		=	=	=	=	=		
Low viscous CMC	4	Low viscosity	=		=		↑	=	↑	=	=		
High viscous CMC	4	High viscosity, poor digestibility	↑		=		=	=	=	=	=		

= indicates no effect, ↑ indicates increased, ↓ indicates reduced.

Table 1 shows an overview how the different diets tested in **Chapter 2 through 5** affected digesta viscosity, digesta moisture content, and excreta and litter quality parameters. Only replacement of 2.5% wheat with coarse grinded oat hulls (insoluble fiber) significantly reduced excreta moisture content (80.9 to 78.8%, $P = 0.035$). It numerically reduced litter moisture content (44.8 to 38.8%, $P = 0.069$) compared with the wheat-based diet by a higher percentage (**Chapter 5**). However, it should be noted that the variation between treatments, expressed by the pooled standard error of the mean, was much higher for litter moisture (2.42) than for excreta moisture (0.75). So even though differences in litter moisture were much larger than differences in excreta moisture, due to large within treatment variation the differences in litter moisture were not significant. In contrast to the effect of coarse oat hulls on excreta and litter moisture, some of the diets negatively affected litter and excreta quality. Feeding a corn-based diet increased excreta (80.9 to 82.9%, $P = 0.044$) and litter moisture content (44.8 to 56.9%, $P < 0.001$) compared with a wheat-based diet at 2 weeks of age (**Chapter 5**). In **Chapter 2**, 6.5% rye was added to the wheat diet with the expectation that it would increase excreta water content. The results showed that at 5 weeks of age there was no significant difference in excreta moisture content between the corn (74.2%) and wheat diet (76.5%). So the additional anti-nutritional factors in rye (Knudsen, 1997) resulted in similar excreta moisture content compared with the excreta of birds fed the corn diet. Adding dietary Mg increased excreta moisture content at 2 and 5 weeks of age by 4.5%, 4.2%, and 7.9% for $MgSO_4$, MgO, and MgCl respectively (**Chapter 4**). Furthermore, digesta viscosity was changed by adding high compared with low viscous carboxymethyl cellulose (**CMC**; 3.87 and 3.16 mPa·s, respectively; $P < 0.001$; **Chapter 5**). The low viscous CMC diet increased excreta moisture content compared with the high viscous CMC diet (83.1 and 81.1%, respectively; $P = 0.049$). Although, litter moisture content was not significantly different between the low (51.9%) and the high viscous CMC diet (46.6%, $P = 0.011$).

Transit time and osmotic value

The observed results of nutritional aspects on excreta moisture content in the different experiments can be related to 2 factors: *i*) increased transit time or *ii*) reduced water reabsorption in the hindgut. The observed effects of the corn and coarse oat hull diet can be related to transit time. Feeding the corn-based diet increased feed intake and water intake compared with the wheat-based diet. However, the water to feed ratio in the corn fed birds

was not different from the wheat fed birds (2.85 and 2.89, respectively; $P = 0.69$). The increased excreta moisture content of the birds fed the corn-based diet may be a consequence of the high average daily feed intake compared with the wheat-based diet (39.4 and 33.2 g, respectively; $P < 0.001$). A higher feed intake was observed to be related to increased passage rate by Almirall and Esteve-Garcia (1994). Also a strong relationship ($r = 0.83$) between transit time and fecal dry weight has been observed by Wiggins (1984). A decrease in transit time results in a higher passage of digesta through the GIT, thereby reducing the time available for reabsorption of water. Along with the increased feed intake, water intake was high (112.4 and 95.9 g, respectively; $P < 0.001$) compared with the wheat diet. A strong relationship between water intake and excreta moisture (Figure 1), as well as between water intake and litter moisture ($r = 0.82$; Ahmad et al., 2009) have been observed previously. The latter result shows that the high feed intake of birds fed the corn diet and the subsequent reduction in GIT transit time was leading the response observed in excreta and litter moisture content.

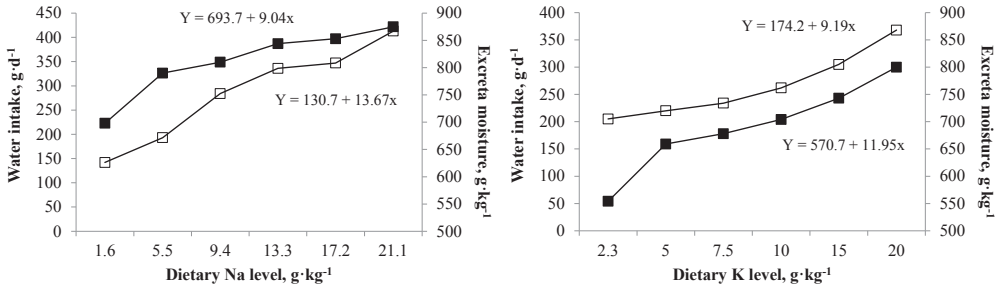

Figure 1. Effect of dietary excess of sodium and potassium on daily water consumption (□) and on excreta moisture content (■) of 38- and 42-week-old laying hens, respectively. Adapted from Smith et al. (2000b).

The positive effect of coarse oat hulls on excreta DM content is thought to be related to transit time also, and more specifically to the transit time in the gizzard. The gizzard regulates particle size in the intestinal tract, thereby controlling the transit time in the GIT. Coarse particles accumulate in the gizzard, increasing the grinding activity of the gizzard (Hetland et al., 2002). The gizzard is composed of muscular tissue. Therefore, increased gizzard activity is thought to result in an increased relative gizzard weight (Svihus, 2011). In

Chapter 5 the relative gizzard weight increased from 2.06 to 2.52% (P = 0.003) when 2.5% coarse oat hulls were added to the wheat diet. Additionally, bile acid concentration in the gizzard increases as a result of increased reflux between the gizzard and the duodenum (Hetland et al., 2003). The digestibility of starch (Hetland et al., 2003; Amerah et al., 2009; Jiménez-Moreno et al., 2013a), CP, and DM (Jiménez-Moreno et al., 2013a) is improved in birds fed coarse oat hulls. The former improvements in nutrient digestibility result in a reduced amount of nutrients in the hindgut. This will reduce osmolality and, subsequently, improve water reabsorption. However, colon digesta moisture was not reduced in birds fed the diet with coarse oat hulls compared with the wheat diet (82.5 and 82.0%, respectively; P = 0.25). No significant difference between the coarse oat hull and wheat diet in average daily water intake (91.3 and 95.9 g, respectively; P = 0.26) or water to feed ratio (2.74 and 2.89, respectively; P = 0.15) were apparent, although they are numerically lower in the birds fed the coarse oat hulls. The latter findings and the absence of a difference in colon moisture content support the transit time effect on excreta and litter moisture content when coarse insoluble fibers were added. Grinding of the oat hulls decreased gizzard development (2.52 to 2.12%; P = 0.009) and increased litter moisture content (38.8 to 46.8 %; P = 0.017) compared with feeding coarse oat hulls. However, no differences in excreta moisture content were observed between the fine and coarse grinded oat hull diets (79.4 and 78.8%, respectively; P = 0.56). Therefore, it can be concluded that, in line with findings by Amerah et al. (2009), insoluble fibers can improve excreta and litter quality, provided that this coincides with a coarse diet.

The relationship between reduced water reabsorption in the hindgut and increased excreta moisture content is shown in **Chapter 4**. Addition of 0.255, 1.02, and 2.04 $g \cdot kg^{-1}$ poorly absorbable ions in the form of different Mg salts to the diet linearly increased digesta and excreta moisture content. The absence of a difference in average daily water intake between the wheat-based diet and the $MgSO_4$ diet in **Chapter 5** (95.9 and 97.5 g, respectively; P = 0.71) supports the hypothesis that the increased excreta moisture content is related to less water reabsorption in the hindgut (Figure 2) and not to increased water intake. Figure 2 presents the digesta moisture content of the different GIT segments. From the duodenum to the distal ileum no differences between the control diet and the different Mg salts were apparent, whereas in the ceca and colon digesta moisture was clearly increased by the Mg salts. Magnesium was not identified by the models in **Chapter 3** to be related to excreta moisture. Latter discrepancy is likely related to the low and fairly constant Mg level in the diets (range of 1.3 to 2.7 $g \cdot kg^{-1}$) fed throughout the experiments in which excreta was

collected, as dietary addition of Mg under practical farming conditions is unusual. In contrast, the experimental dietary levels ranged from 1.9 to 4.2 $g \cdot kg^{-1}$. With a linear increase of excreta moisture content and increased dietary Mg inclusions, the experimental Mg inclusion levels showed a strong response in excreta moisture content particularly at the higher dietary levels.

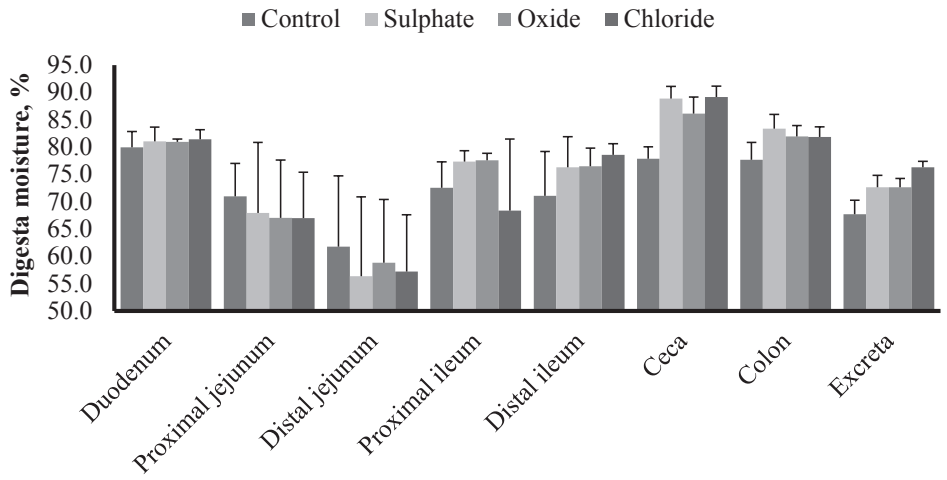

Figure 2. Percentage of digesta moisture throughout the different intestinal segments at 36 days of age from birds fed a control diet (no added Mg) or 2.04 $g \cdot kg^{-1}$ Mg supplied via $MgSO_4$ (Sulphate), MgO (Oxide), or MgCl (Chloride).

In **Chapter 3**, we considered the nutrient content in the excreta as a representative parameter for the osmotic load in the hindgut. The results clearly indicate important relationships between minerals and excreta moisture output, where greater levels of Zn and K and the interactions between contents of NDF × K, NDF × crude protein, and Ca × P resulted in lower excreta moisture levels. In contrast, greater levels of Na, P, Ca, and crude protein content and the interaction between contents of NDF × Na, NDF × Zn, and K × Cu resulted in greater excreta moisture levels. These results are in line with previous observations (Murakami et al., 1997; Smith et al., 2000b; Oviedo-Rondón et al., 2001; Borges et al., 2003b; Jankowski et al., 2011). Increased dietary Na (Smith et al., 2000b; Ahmad et al., 2009) and P contents (Smith et al., 2000b) linearly increase water intake. Additionally, higher nutrient excretion is indicative of increased osmolality of the digesta in the hindgut. Especially high mineral levels, which was concluded from high mineral levels in the excreta, will prevent water reabsorption in the hindgut. In pigs, mineral contents of feces have been identified as

main osmotic particles (Etheridge et al., 1984). If the osmotic gradient between the digesta and the blood serum increases, this will result in additional water flow towards the gut lumen to restore the osmotic balance. Consequently, water output via the excreta will increase (Vena et al., 1990; Smith et al., 2000b).

Also the interactive effects of minerals (e.g., Na and Zn) with NDF, which increased excreta moisture, may be explained by ion-exchange properties of insoluble dietary fibers. The binding properties of the fibers is dependent on the concentration of the minerals (Laszlo, 1987). When the Na and Zn are bound to dietary fibers, their absorption in the GIT is potentially prevented. As a consequence, the osmotic load in the digesta will increase and result in water moving into the gut lumen. Furthermore, residual fiber fractions may still have a water binding capacity, increasing water output into the excreta (Armstrong et al., 1993). However, available data are limited to in vitro studies and additional experiments are required to further explore these interactions.

In summary, the results discussed show that with nutrition excreta moisture and litter moisture content can be manipulated. The effects of nutrition are related to nutrient digestibility and subsequent nutrient output, although transit time is also an important factor determining excreta moisture output. The effects on litter moisture content followed the trend in excreta moisture content, where higher excreta moisture content resulted in higher litter moisture content and vice versa. These results show the value of nutritional aspects in changing excreta moisture output and, subsequently, improving litter quality.

Microbiota in relation to excreta moisture content

Zinc reduced excreta moisture content (**Chapter 3**), which is in line with its well-known effect in reducing diarrhea in piglets (Fairbrother et al., 2005) and humans (Patel et al., 2011). The proposed mechanism is either via inhibition of Cl secretion and enhancement of Na absorption from the lumen, which prevents water excretion caused by osmotic differences between serum and digesta (Hoque et al., 2009). Another possibility is via effects of Zn on microbial composition and activity throughout the intestinal tract (Højberg et al., 2005). The microbiota ferment undigested nutrients in the hindgut, producing compounds such as short chain fatty acids (**SCFA**; Skadhauge and Holtug, 1993; Kelly and King, 2001). Fermentation can directly improve water reabsorption by lowering the nutrient content of the digesta or indirectly by SCFA-linked Na and water reabsorption (Rice and Skadhauge, 1982). However,

the results of **Chapter 2** show that the differences in nutrient absorption by the bird did not result in a significant change in bacterial composition. The medium chain fatty acid (**MCFA**) diet however did cause changes in microbiota composition. Additionally, none of the dietary treatments significantly affected moisture output via the excreta. The results of **Chapter 2** and findings by Choct et al. (2010) question the significance of microbial fermentation in fast growing birds. They have a high feed intake and thus a high rate of digesta passage. Passage rate of digesta is a method to control the level of luminal bacteria by the host, as bacteria not adhered to the mucus will be excreted with the digesta (Oli et al., 1998). It was also suggested by Thompson et al. (2008) that the transit time of digesta may limit the possibility of the luminal bacteria to adapt to the nutrient level changes.

The effect of the MCFA added to the diet on feed efficiency coincided with a change in bacterial composition in the ileum (Table 2). These changes in bacteria species appeared positive for the birds' energy balance, leading to improved growth. However, in the experiment the overall performance of the birds was low, suggesting a possible presence of a subclinical infection. Therefore, the improved performance may have also resulted from a direct antibacterial effect of the MCFA (Kabara et al., 1972; Skřivanová et al., 2005) or an indirect effect via the immune system (Lee et al., 2001). It would be interesting to evaluate if birds fed MCFA have a higher resistance to pathogen challenge. A pH below 4.0 inhibits in vitro growth of pathogens and putrefactive bacteria by 50% (Bruno and Shah, 2002). Feeding the MCFA diet lowered ceca pH, which may indeed limit fermentation and reduce the production of enterotoxin compounds by putrefactive bacteria. However, the pH reduction was less significant (6.44 to 6.19) compared with the in vitro reduction observed by (Bruno and Shah, 2002), and the effects of lowering pH in vivo warrants additional study.

Little knowledge is available on the effect of specific commensal bacterial species on bird production performance. Some relations between growth depression, poor feed efficiency and specific bacteria such as *Entercococcus hirae* (Chadfield et al., 2005), *Lactobacillus salivarius* (Guban et al., 2006), and a specific taxonomic unit, potentially representing *L. salivarius*, *L. avaiarius*, and *L. crispatus* (Torok et al., 2011) were previously described. However, different techniques (e.g., culturing or molecular fingerprinting) have been used to evaluate the microbial composition, limiting the possibilities for comparison between experiments (Gong et al., 2002; Hübener et al., 2002; Knarreborg et al., 2002). Knowledge about what a 'healthy' microbial composition is composed of is important when studying changes in antibiotic use. As we can modify the microbial composition by diet (**Chapter 2**,

Hübener et al., 2002; Hetland et al., 2004; Santos et al., 2007; Rehman et al., 2009), there are opportunities to steer the bacterial species present in the GIT and promote a healthy and stable gut environment. A stable gut flora makes the bird resilient towards pathogen invasion subsequently reduces the need for antibiotic treatment.

Table 2. Overview taxonomic coverage that are significantly up- or down-regulated in the birds fed the diet supplemented with medium-chain fatty acids compared with the other 4 diets (corn, 15% resistant starch, wheat, and wheat supplemented with an enzyme)

Up-regulated in MCFA group	Down-regulated in MCFA group
Enterobacteriaceae; Citrobacter spp.	*Lactobacillaceae; Lactobacillus panis/ponti*
Enterobacteriaceae; Escherichia coli, Shigella	*Listeriaceae; Listeria* sp.
Enterobacteriaceae; Enterobacter group	*Lactobacillaceae; Lactobacillus* unclassified
Enterobacteriaceae; E. coli/Shigella	*Lactobacillaceae; Pediococcus* spp.
Enterobacteriaceae; Enterobacter unclassified	*Lactobacillaceae; Lactobacillus jensenii/salivarius*
Enterobacteriaceae; Escherichia coli, Shigella	*Micrococcaceae, Rothia mucilaginosa/nasimuri*
Enterobacteriaceae; Enterobacter unclassified	*Lactobacillaceae; Lactobacillus* group
Lactobacillaceae; Lactobacillus reuteri	*Lactobacillaceae; Lactobacillus* spp.
Lactobacillaceae; Lactobacillus	*Enterococcaceae*
Lactobacillaceae; Lactobacillus	*Lactobacillaceae; Lactobacillus*
Lactobacillaceae; Lactobacillus	*Lactobacillaceae; Lactobacillus*
Lactobacillaceae; Lactobacillus reuteri	

A limitation of the array technique used to measure the microbiota composition is the absence of a measure of total bacteria numbers. The composition as such was not affected, although the number of bacteria may have been increased with higher nutrient levels in the digesta (Hübener et al., 2002; Rodríguez et al., 2012). It is also possible that even though changes in ileal luminal bacteria species were limited, mucosal associated bacterial species were affected by the different nutrient content of the diets tested (Uni et al., 2005; Thompson et al., 2008). Finally, the differences in nutrient digestibility may not have changed the bacterial species in the ileal digesta, whereas ceca microbiota may have been subject to microbial changes. Short chain fatty acid production by bacteria is much higher in the ceca compared with the ileum (e.g., 1,464 and 335 $mg \cdot kg^{-1}$, respectively), and more prone to dietary changes (1,464 and 3,652 in corn and wheat/rye fed birds, respectively; Hübener et al., 2002). The results show that the ceca are the main fermentation segments. Future studies on the effect of diet on microbial changes should therefore include measurements of luminal and mucosal-associated bacterial populations of the ileum and ceca, as well as a measure for total bacteria levels to be able to fully evaluate dietary effects on intestinal microbiota. However,

even if changes in bacteria counts or in ceca microbiota were present it still did not result in significant changes in excreta moisture excretion by the birds.

In conclusion, based on the results of **Chapter 2** there seems to be limited relation between variations in commensal bacteria in birds fed different practical dietary compositions and excreta quality. In humans, a similar observation has been reported (Ikeda et al., 1994). Even though the balance between the host and its endogenous bacteria seems delicate, the results of **Chapter 2** suggest that only a disturbance with pathogens could induce bacterial diarrhea. However, the composition of the diet (ingredient and nutrient levels) can predispose the host to GIT disturbances, providing opportunity for enteropathogens like *Escherichia coli* and *Clostridium perfringens* to invade the GIT epithelial layer. Therefore, it is important to have a rather stable flora that is effective in competitive exclusion of pathogens. This will prevent overgrowth and invasion by pathogens. One limitation is knowledge about the composition of a 'healthy' commensal flora. Although, a wide range in microbial composition is reported in literature without causing detrimental effects to the host. The relation between commensal bacterial flora composition and excreta moisture output seems limited and more related to pathogen invasion and gut integrity.

MEASURES TO ASSESS EXCRETA AND LITTER QUALITY

Solely total moisture level may not be sufficient to describe the quality of the excreta and litter. In the digesta, excreta, and litter water can be present in a free form, bound to solutes (e.g., protein, fibers, and electrolytes), or captured within capillary structures. The strength of the water binding is depended of the type of bond e.g., ionic, covalent, hydrogen, or enclosure in capillaries (Chaplin, 2003). As more functional alternatives to total moisture content, such as free water (**Chapter 4 and 5**) and water activity ($\mathbf{A_w}$; **Chapter 5**) were evaluated to assess water content in excreta and litter. Free water indicates the mechanical properties of water in a sample and includes the water entrapped in capillaries, although, its value is depending on the g-force applied during centrifuging. In contrast, A_w is a measure for the thermodynamics of water in a sample, related to the escaping tendency of water (Agoda-Tandjawa et al., 2013). It indicates the available water component (Opara et al., 1992) that correlates with microbial growth (Scott, 1957).

The results of **Chapter 5** show only small effects between different treatments on excreta and litter A_w. The latter finding is likely related to the high values of A_w measured, all

close to the maximum A_w value of 1 (range 0.978 to 0.994). Only at A_w values below 0.80 bacterial growths starts to decline. For Salmonella, no growth inhibition has been observed at excreta A_w levels above 0.85, whereas A_w levels below 0.85 reduced growth (Himathongkham et al., 1999; Hayes et al., 2000; Payne et al., 2007). With the mean A_w value of 0.99 for both litter and excreta values observed, no effect on microbial activity was expected. In commercial poultry barns approximately 75.0% of the litter samples have A_w values above 0.90 and moisture contents higher than 30.0% (Hayes et al., 2000). The high A_w values observed are likely related to the high moisture content of the samples (mean value of 80.9% and 47.4% for excreta and litter, respectively). There is a strong relation between moisture and A_w (Himathongkham et al., 1999; Hayes et al., 2000) and only at a litter moisture content below 26.0% A_w decreases rapidly (Hayes et al., 2000). These findings indicate measuring A_w is less suitable for high moisture substrates like excreta and litter.

In contrast to the absence of a response to A_w, free water was affected by changing jejunum viscosity and by adding a laxative to the diet. Excreta free water content increased from 18.3 to 30.8% when feeding a corn-based diet compared with feeding a wheat-based diet. Similarly, feeding low viscous compared with high viscous CMC increased excreta free water content from 19.9 to 29.9% (**Chapter 5**). The lower excreta free water content in the birds fed the wheat-based and the high viscous CMC diet coincided with a higher jejunum digesta viscosity compared with the corn-based (2.56 and 1.89 mPa·s, respectively; $P < 0.001$) and low viscous CMC diet (2.59 and 3.17 mPa·s, respectively; $P < 0.001$). This is potentially a result of the water being trapped in a gel-matrix in the more viscous digesta (Hetland et al., 2004) preventing release of the water upon centrifuging force.

The binding of the water in the excreta of the birds was also affected by the dietary Mg source, as shown by the differences in percentage free water in the excreta (Figure 3; **Chapter 4**). Compared with the control diet (no added Mg) adding MgO in the diet increased free water on average 4.9 times, $MgSO_4$ 5.8 times, and MgCl 10.5 times. The increase in free water content in response to 0.255, 1.02, and 2.04 $g \cdot kg^{-1}$ Mg addition was linear for all 3 Mg sources, although in increase in the MgCl treatment was stronger (slope 11.2) compared with the other 2 Mg sources tested (slopes 6.9 and 5.4 for $MgSO_4$ and MgO, respectively; Figure 3). In line with previous observations, 2.04 $g \cdot kg^{-1}$ Mg supplied by $MgSO_4$ had no effect on total excreta moisture content compared with the wheat-based control diet (80.9 and 81.1%, respectively; $P = 0.85$), although it increased excreta free water content from 18.3 to 25.8% ($P = 0.015$) in **Chapter 5**. These results indicate that free water measurement provides a

potential different quality measurement for excreta moisture content. However, free water could not be determined in litter samples. This is probably related to the relatively lower moisture content of litter or more fibrous structure of the litter material, which makes free water unsuitable for litter samples.

In conclusion, solely water content does not provide any information about the status of the water in the sample. The results of **Chapter 5** indicate that, even though A_w is a more precise measure for available water, the high moisture content of excreta and litter samples limits the applicability of A_w as a qualitative measurement. Free water, even though this parameter is dependent of the centrifugal speed applied, seems more suitable as qualitative measurement for excreta samples (**Chapter 4 and 5**). However, more in-depth study towards to effects on water reabsorption in the distal GIT and evaporation from low and high free water excreta samples is required to estimate its importance for practical implications.

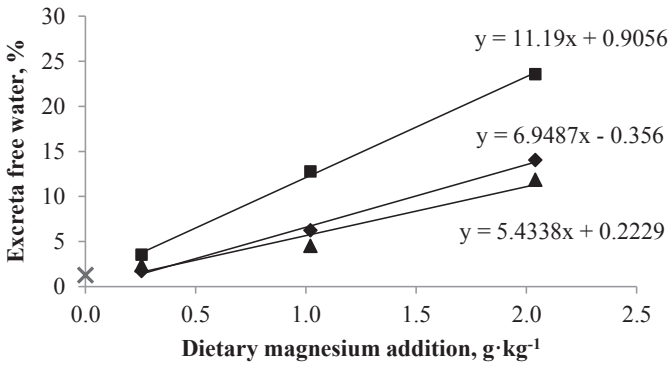

Figure 3. Percentage of excreta free water at 36 d of age from birds fed the control diet (no dietary Mg addition; ×) or fed increasing dietary levels of $MgSO_4$ (♦), MgO (▲), and MgCl (■).

CONCLUSIONS

The observed results of nutritional aspects on excreta moisture content in the different experiments could be related to 2 factors: *i*) increased transit time or *ii*) reduced water reabsorption in the hindgut. A decrease in transit time results in a higher passage of digesta through the gastrointestinal tract, thereby reducing the time available for reabsorption of water. Insoluble fibers, provided that this coincides with a coarse diet, can be used to slow

down transit time and optimize digestibility, thereby improving excreta and litter quality. Adding poorly absorbable ions (e.g., Mg) linearly increase digesta and excreta moisture content by reduced water reabsorption in the hindgut. Minerals and other undigested nutrients will increase the osmotic load of the digesta in the hindgut and result in water moving into the gut lumen. The results discussed in this chapter show the value of nutritional aspects in changing excreta moisture output and, subsequently, improving litter quality.

The results of **Chapter 2** question the significance of microbial fermentation in fast growing birds, with a high feed intake and, consequently, high digesta passage rate. The transit time of digesta seems to limit the possibility of the luminal bacteria to adapt to the nutrient level changes. Additionally, there is a limited relation between variations in luminal commensal bacteria and excreta quality. However, the composition of the dietary ingredients and nutrient levels can predispose the host to disturbances, providing opportunity for enteropathogens to invade the epithelial layer.

Reducing excreta moisture content via nutrition provides a good means to reduce litter moisture in apparently healthy birds, although it is also depending on the amount of excreta produced by the birds. When evaluating excreta quality, solely water content does not provide any information about the status of the water in the sample. Water activity can be used as a predictor for microbial growth. However, its use is limited in high moisture (> 30.0%) content excreta and litter samples. Free water, even though dependent of the centrifugal speed applied, can be used in high moisture samples to assess water binding in the sample. A limitation of this measurement is its use in relatively lower moisture content litter samples.

IMPLICATIONS IN A BROADER PERSPECTIVE

Commercial broiler grower profits are under pressure due to high feed prices and low revenues. Additionally, increasing demands for welfare resulted in a reduction of the stocking density allowed during grow-out. On the backside, there is an increasing demand for protein to feed the growing human population. The FAO predicted a 25% increase of per capita meat consumption from 2000 to 2030, related to increased protein consumption of developing countries. It will be challenging to meet this growing demand for animal protein and animal production will have to increase to adequately feed future population. To increase production, bird selection has focused on high growth rates and efficiency (Willems et al., 2013). However, this high growth rate also made them more susceptible for metabolic and health

disorders (Rauw et al., 1998). With the selection for growth, also the volume of feed consumed increases. However, as shown in this thesis, a high feed intake leads to a high throughput of digesta and a higher production of excreta that contains more moisture. Current feed strategies need to aim at optimizing the gastrointestinal tract functions via the diet. As shown in this thesis, many nutritional factors that lead to high excreta moisture output can be related to transit time or reduced water reabsorption in the hindgut. Therefore, nutritionist should use the knowledge generated in this thesis to optimize dietary formulations. Stimulation of the gizzard seems an effective way to slow down passage rate and optimize nutrient digestibility. Optimizing digestibility, thereby reducing the output of those nutrients identified in this thesis to increase moisture output, will reduced the risk of dietary related wet litter incidences. At the same time, a proper functioning digestive tract and optimal digestibility will reduce the risk for microbial disturbances in the hindgut, thereby reducing the need for antibiotic treatment.

Reducing moisture and nutrient content of the excreta will also have an impact on the environment. Improved efficiency reduces the impact of poultry on the environment (Willems et al., 2013). Improving digestibility will reduce nutrient and water output via the excreta and, subsequently, reduce the run-off of nutrients during land disposal. Additionally, ammonia and odor emission will be reduced in drier poultry houses. Therefore, besides assessment of litter quality, also monitoring excreta quality throughout the growing period is highly recommended for managing litter quality, broiler health, and environmental impact. This should not be limited to measuring total excreta moisture, as the results of this thesis show that the status of the water in the excreta can be different. This status may be more important considering microbial activity in the litter, ammonia production and emission, and litter drying properties (e.g., evaporation).

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Summary

In commercial poultry farming, broilers are usually housed on litter, primarily composed of bedding material (e.g., wood shavings) mixed with feed, feathers, and excreta throughout the grow-out period. Litter in poultry barns can vary greatly in moisture content, ranging between 15 and 45%. When the amount of water added to the litter (e.g., from excreta and water spillage) exceeds the amount of water evaporated, litter moisture will increase and a condition termed "wet litter" can occur. Wet litter is used as a term to describe a poor condition of the litter in poultry barns, defined as "*when material covering the floors of poultry houses reaches its saturation threshold and is unable to hold more moisture*". Commonly, wet litter is observed when litter moisture concentration is higher than 25 to 35%, depending on the type of litter material used. Wet litter causes more ammonia to be produced by bacteria, which is emitted into the air and can negatively affect animal production and welfare. Wet litter problems in poultry have been reported already since 1950. In general, the incidence of wet litter increases with more intensively housing of poultry. In the UK, an incidence rate of up to 75% has been reported in 2001. Wet litter is considered a multifactorial problem, with management and housing of the birds, disease control, diet, and gut health being the main factors involved. In one-third of the cases more than one factor can be identified to be associated with wet litter. The abovementioned factors contribute to either a too high input of moisture onto the litter or a too low output of moisture from the litter. Improvements in nutrient digestibility from feed and improved gut health appear to be major factors to reduce wet litter incidences.

The microbiota has an important metabolic function including fermentation of undigested compounds in the hindgut. By changing the type or level of dietary nutrients that reach the hindgut the bacterial diversity and digesta composition can potentially be altered. In Chapter 1, the effect of 5 different diets, varying in dietary ingredients (medium-chain fatty acids, nonstarch polysaccharides, and starch) on the microbiota composition of ileal digesta of broiler chickens and on excreta dry matter content were studied. Each treatment was repeated 6 times in cages each containing 18 Ross 308 broilers, with growth performance measured from 0 to 34 d of age and excreta dry matter and ileal microbiota composition analyzed at 34 d of age. Microbiota composition was evaluated using a novel ribosomal RNA microarray technology containing 370 different probes covering various genera, groups of microbial species, and individual species of the chicken gut microbiota. The results showed that the differences in nutrient absorption by the bird did not result in significant changes in bacterial composition. However, feeding a diet with medium-chain fatty acids (0.3% C10 and 2.7%

C12) improved feed efficiency compared with the other dietary treatments, which coincided with significant changes in bacterial composition in the ileum digesta. Gram-positive bacteria belonging to the phylum of the *Firmicutes*, including *Lactobacillus* species, and species belonging to the family of the *Enterococcaceae* and *Micrococcaceae* were suppressed, while the gram-negative bacteria belonging to the family of the *Enterobacteriaceae* were promoted. However, excreta moisture output by the birds was not affected by any of the dietary treatments.

In Chapter 2, the objective was to study the relationship between nutrient contents and the moisture content of the excreta of broilers. Excreta nutrient and moisture contents of samples were collected throughout 8 different broiler feeding trials. A dataset containing 351 observations, originating from 2008 (n = 178) and 2009 (n = 173) and collected at 6 (n = 6), 17 (n = 24), 28 (n = 36), and 35 d of age (n = 285), was used. The final data set of potential predictor variables contained 78 variables, including all main effects and their squared terms and first order interactions which were considered nutritionally relevant. Using a biological based model approach following the general form $Y = X\beta + Zu + e$, a model with 10 variables ($R^2 = 0.54$) and another one with 14 variables ($R^2 = 0.58$) were developed. Subsequently, these models were compared with a statistical model, in which all potential independent variables were recursively tested for their contribution, to check for omissions of variables that had significant contributions. The models identified important relationships between nutrients and excreta moisture output, where greater levels of Zn and K and the interactions between contents of NDF × K, NDF × protein, and Ca × P resulted in lower excreta moisture levels, whereas greater levels of Na, P, Ca, and protein content and the interaction between contents of NDF × Na, NDF × Zn, and K × Cu resulted in greater excreta moisture levels. These models confirmed the effect of minerals on excreta moisture content. Furthermore, hitherto unknown nutrient interactions that contribute to excreta moisture level were identified that may provide new insights into the multifactorial problem of wet litter in poultry production.

Dietary mineral levels affect intestinal conditions with regard to osmolality and water reabsorption, where Mg is often used as an osmotic laxative. Under practical farming conditions dietary addition of Mg is unusual, and the Mg level in diets is usually low and fairly constant (1.3 to 2.7 $g \cdot kg^{-1}$). The objective of Chapter 3 was to evaluate Mg in broiler diets as a wet litter model for reduced intestinal water reabsorption and increased excreta moisture level. Effects of Mg source (magnesium sulfate, magnesium oxide, and magnesium

chloride), each added at 3 levels (0.255, 1.02, and 2.04 $g \cdot kg^{-1}$ diet), were studied. The former additions resulted in dietary Mg contents ranging from 2.2 to 3.9 $g \cdot kg^{-1}$ diet. The 10 treatments were randomly assigned to cages within 6 blocks, resulting in 6 replicates per treatments with 18 birds per replicate. Adding Mg to the diet of broilers linearly increased the excreta moisture and excreta free water content. Compared with the control diet (no added Mg) adding MgO increased excreta moisture by 4.2%, MgSO4 by 4.5%, and MgCl by 7.9%. Additionally, adding MgO in the diet increased free water on average 4.9 times, MgSO4 5.8 times, and MgCl 10.5 times compared with the control diet. The increase of excreta free water in the MgCl treatment was stronger (slope 11.2) compared with the other 2 Mg sources tested (slopes 6.9 and 5.4 for MgSO4 and MgO, respectively). This rejects the hypothesis that MgO and MgCl are less laxative sources compared with $MgSO_4$. From the duodenum to the distal ileum no differences between the control diet and the different Mg salts were apparent, whereas in the ceca and colon digesta moisture was clearly increased by the Mg salts. This increase in digesta moisture percentage supports the hypothesis that Mg changes water reabsorption in the distal gut segments. The later finding confirms that dietary Mg addition can be used as a model to study wet litter caused by reduced intestinal water reabsorption.

In Chapter 4, the effects of 8 different dietary factors, including nonstarch polysaccharide content (corn versus wheat), particle size of insoluble fiber (coarse versus fine grinded oat hulls), viscosity of a non-fermentable fiber (low and high-viscous carboxymethyl cellulose), addition of a clay mineral (sepiolite), and a laxative ($MgSO_4$) on litter and excreta moisture content were investigated. Additionally, free water and water activity (A_w) of the excreta were evaluated as alternative excreta and litter quality parameters. The 8 treatments were randomly assigned to cages within blocks, resulting in 12 replicates per treatments with 6 birds per replicate. The dietary treatments had limited effects on excreta or litter A_w. Increasing dietary nonstarch polysaccharide content by feeding a corn-based diet (low NSP) compared with feeding a wheat-based diet (high NSP) increased excreta and litter moisture content. Gastrointestinal transit time of diet/digesta may have affected moisture intake and excretion. Addition of coarse oat hulls (insoluble fiber) reduced excreta and litter moisture content and litter A_w. Grinding of the oat hulls decreased gizzard development and increased litter moisture content compared with feeding coarse oat hulls. Therefore, it can be concluded that insoluble fibers can improve excreta and litter quality, provided that this coincides with a coarse diet. The effects of changing viscosity or adding a clay mineral or laxative deviated from results observed in previous studies. It was concluded that the measurement A_w does not

add to our understanding of moisture in litter and excreta, as all values were close to 1.0. Therefore, measurement of total moisture and free water content of excreta are more descriptive and valuable.

The main conclusions from this thesis are *i*) there seems to be a limited relationship between differences in commensal bacteria in healthy birds fed practical dietary compositions and excreta quality, *ii*) dietary formulations can be adjusted with the outcomes of the models from Chapter 2 to change levels of nutrients excreted and, consequently, reduce moisture output, *iii*) Mg addition in the diet can be used as a model to study wet litter caused by reduced intestinal water reabsorption, *iv*) effects of insoluble fibers on excreta moisture is dependent of particle size, and *v*) excreta free water is a more suitable measure to asses excreta quality than A_w. The implication of the measurement of excreta free water on litter conditions in broiler houses warrants further investigation.

Samenvatting

In de commerciële pluimveehouderij worden vleeskuikens voornamelijk gehuisvest op strooisel, bestaande uit beddingmateriaal (bijv. houtkrullen), wat zich vermengt met voer, veren en mest gedurende de mestperiode. Het vochtgehalte van het strooisel tijdens de mestperiode in commerciële pluimveestallen kan sterk variëren, waarbij waardes tussen 15 en 45% vocht zijn waargenomen. Wanneer de hoeveelheid water toegevoegd aan het strooisel (bijv. via mest en morsen van water) groter is dan de hoeveelheid water die verdampt zal het vochtgehalte van het strooisel toenemen en kan een conditie genaamd "nat strooisel" optreden. Nat strooisel wordt gebruikt als een term om een slechte staat van het strooisel in pluimveestallen te beschrijven, gedefinieerd als 'wanneer het beddingmateriaal in pluimveestallen haar verzadigingsniveau bereikt heeft en niet in staat is om meer vocht vast te houden'. Gewoonlijk wordt natte mest waargenomen wanneer het vochtgehalte in het strooisel hoger is dan 25 tot 35%, afhankelijk van het type beddingmateriaal. Natte mest veroorzaakt een toename van ammoniak productie door bacteriën, die wordt uitgestoten in de lucht en kan een negatieve invloed hebben op technische resultaten en dierenwelzijn. Reeds in 1950 werd melding gemaakt van nat strooisel problematiek in pluimvee. In het algemeen is de incidentie van nat strooisel toegenomen met de intensievere huisvesting van pluimvee. Het Verenigd Koninkrijk rapporteerde een incidentie van 75% nat strooisel gevallen in de pluimveehouderij in 2001. Nat strooisel wordt beschouwd als een multifactorieel probleem, waarbij het management en huisvesting van de dieren, ziektebestrijding, voeding en darmgezondheid de belangrijkste factoren zijn. In een derde van de gevallen kan meer dan één factor worden geïdentificeerd die is geassocieerd met nat strooisel. Bovengenoemde factoren dragen bij aan ofwel een te hoge toevoeging van vocht aan het strooisel of een te lage verdamping van vocht uit het strooisel. Verbeteringen in nutriëntverteerbaarheid van het voer en betere darmgezondheid lijken belangrijke factoren om nat strooisel incidenten te verminderen.

De darmflora vervult een belangrijke metabolische functie door middel van fermentatie van onverteerde nutriënten in de dikke darm. Door het veranderen van het type of het niveau van voedingsstoffen die de dikke darm bereiken, kan de diversiteit van de bacteriën en samenstelling van de darminhoud mogelijk worden gewijzigd. In hoofdstuk 1 is het effect van 5 verschillende voeders, variërend in ingrediënten (middellange keten vetzuren, niet-zetmeel polysachariden en zetmeel) op de darmflora samenstelling van de darminhoud in ileum en het droge stof gehalte in vleeskuikenmest onderzocht. Elke behandeling werd 6 keer herhaald in hokken met elk 18 Ross 308 vleeskuikens. De groei werd gemeten van 0 tot 34 d

leeftijd en op 34 d leeftijd is het droge stof gehalte van de mest en de samenstelling van de darmflora in het ileum geanalyseerd. De samenstelling van de darmflora werd geëvalueerd met behulp van een nieuwe ribosomaal RNA microarray technologie met 370 verschillende probes die verschillende geslachten, groepen van micro-organismen en individuele soorten van de kippendarmflora vertegenwoordigen. De resultaten toonden aan dat de meeste verschillen in opname van voedingsstoffen door het vleeskuiken niet resulteerde in significante veranderingen in bacteriële samenstelling. Echter, het voer met middellange keten vetzuren (0,3% C10 en 2,7% C12) verbeterde de voederconversie in vergelijking met de andere voederbehandelingen, welke samenviel met significante veranderingen in de bacteriële samenstelling van de darminhoud van het ileum. Gram-positieve bacteriën die behoren tot het phylum van de *Firmicutes*, zoals *Lactobacillus* soorten en soorten van de familie van de *Micrococcaceae Enterococcaceae* werden onderdrukt, terwijl de Gram-negatieve bacteriën die behoren tot de familie van de *Enterobacteriaceae* werden bevorderd. Echter, het vochtgehalte van de mest van vleeskuikens werd niet beïnvloed door een van de voerbehandelingen.

In hoofdstuk 2 is de relatie tussen nutriënten gehaltes en het vochtgehalte van de mest van vleeskuikens geëvalueerd. De nutriënt- en vochtgehaltes van de mestmonsters werden gemeten gedurende 8 verschillende voerexperimenten met vleeskuikens. Voor de analyse werd een dataset gebruikt met 351 waarnemingen, afkomstig uit 2008 (n = 178) en 2009 (n = 173) en verzameld op 6 (n = 6), 17 (n = 24), 28 (n = 36) en 35 d leeftijd (n = 285). De uiteindelijke dataset van potentiële voorspellende variabelen bevatte 78 variabelen, waaronder alle hoofdeffecten, hun kwadratische effecten en eerste orde interacties die als nutritioneel relevant werden beschouwd. Met behulp van een op biologie gebaseerd model, met de algemene vorm $Y = X\beta + Zu + e$, werden een model met 10 variabelen ($R^2 = 0,54$) en een met 14 variabelen ($R^2 = 0,58$) ontwikkeld. Vervolgens zijn deze modellen vergeleken met een statistisch model, waarin alle potentiële onafhankelijke variabelen recursief zijn getest op hun eventuele significante bijdrage, om te controleren of er mogelijk variabelen ontbraken in de biologische modellen. De modellen identificeerden verschillende belangrijke relaties tussen nutriënten en vochtgehalte van de mest, waar een hoger gehalte Zn en K en de interacties tussen de gehaltes van NDF × K, NDF × ruw eiwit en Ca × P resulteerde in een lager vochtgehalte in de mest, terwijl een hoger gehalte van Na, P, Ca en ruw eiwit en de interactie tussen de gehaltes van NDF × Na, NDF × Zn en K × Cu resulteerde in een hoger vochtgehalte in de mest. Deze modellen bevestigde het effect van mineralen op het vochtgehalte in de mest.

Bovendien werden tot nu toe onbekende nutriënt interacties geïdentificeerd, die bijdragen aan het vochtgehalte van de mest en die nieuwe inzichten kunnen verschaffen in de multifactoriële problematiek van natte mest in de pluimveehouderij.

Mineraalgehaltes van het voer beïnvloeden de condities in de darm met betrekking tot osmolariteit en waterabsorptie, waarbij Mg vaak gebruikt wordt als een osmotisch laxeermiddel. In de pluimveehouderij is toevoeging van Mg ongebruikelijk en is het Mg-niveau in het dieet meestal laag en redelijk constant (1,3-2,7 $g \cdot kg^{-1}$). Het doel van hoofdstuk 3 was het evalueren van Mg toevoeging in het voer van vleeskuikens als een natte mest model voor verminderde waterabsorptie in de darm en verhoogd vochtgehalte in de mest. De effecten van 3 verschillende Mg bronnen (magnesiumsulfaat, magnesiumoxide en magnesiumchloride) werden getest, elk op 3 niveaus (0,255, 1,02 en 2,04 $g \cdot kg^{-1}$). Deze toevoegingen resulteerde in Mg gehaltes in de voeders tussen 2,2-3,9 $g \cdot kg^{-1}$. De 10 behandelingen werden willekeurig toegewezen aan een hok binnen 6 blokken, resulterende in 6 herhalingen per behandeling met 18 Ross 308 vleeskuikens per hok. Het toevoegen van Mg aan het dieet van vleeskuikens resulteerde in een lineaire toename in vochtgehalte en vrij watergehalte van de mest. In vergelijking met het controle dieet (geen toegevoegde Mg) verhoogde toevoegen van MgO het vochtgehalte in de mest met 4,2%, MgSO4 met 4,5% en MgCl met 7,9%. Daarnaast resulteerde het toevoegen van MgO in gemiddeld 4,9 maal hoger vrij water, MgSO4 5,8 maal en MgCl 10,5 maal vergeleken met het controle dieet. De toename van vrij water in de mest voor de MgCl behandeling was sterker (helling 11,2) in vergelijking met de 2 andere Mg bronnen getest (hellingen 6.9 en 5.4 voor respectievelijk MgSO4 en MgO). Dit verwerpt de hypothese dat MgO en MgCl minder laxerend zijn in vergelijking met MgSO4. Van de twaalfvingerige darm tot het einde van het ileum waren er geen verschillen tussen het controle dieet en de verschillende Mg bronnen op het vochtgehalte van de darminhoud, terwijl in de blinde en dikke darm het vochtgehalte van de darminhoud duidelijk verhoogd was door het toevoegen van Mg. Deze toename van het vochtpercentage van de darminhoud ondersteunt de hypothese dat Mg de waterabsorptie aan het einde van het maagdarmkanaal vermindert. Deze bevinding bevestigt dat toevoeging van Mg aan het voer kan worden gebruikt als model om natte mest, veroorzaakt door verminderde waterabsorptie in de darm, te bestuderen.

In hoofdstuk 4 zijn de effecten van 8 verschillende voedingsfactoren, waaronder niet-zetmeel polysacharide gehalte (maïs versus tarwe), deeltjesgrootte van onoplosbare vezels (grof versus fijn gemalen haverdoppen), viscositeit van een niet-fermenteerbare vezel (laag-

en hoog- viskeuze carboxymethyl cellulose), toevoeging van een kleimineraal (sepioliet) en een laxeermiddel (MgSO4) op het vochtgehalte van strooisel en mest onderzocht. Daarnaast zijn vrij water en wateractiviteit (A_w) van de mest en het strooisel geëvalueerd als alternatieve kwaliteits-parameters. De 8 behandelingen werden willekeurig toegewezen aan hokken binnen blokken, resulterend in 12 herhalingen per behandeling met 6 dieren per herhaling. De voerbehandelingen hadden beperkte effecten op mest en strooisel A_w. Het verhogen van het niet-zetmeel polysacharide gehalte in het voer, door middel van een mais dieet (laag NSP) in vergelijking met een tarwe dieet (hoog NSP), resulteerde in een toename van het vochtgehalte in mest en strooisel. De doorloopsnelheid in het maagdarmkanaal van het dieet / darminhoud kan de water- opname en uitscheiding hebben beïnvloed. Toevoeging van grove haverdoppen (onoplosbare vezels) resulteerde in een lager vochtgehalte en A_w van de mest en het strooisel. Fijn malen van de haverdoppen verminderde spiermaag ontwikkeling en verhoogde het vochtgehalte van het strooisel in vergelijking met het voeren van grove haverdoppen. Derhalve kan worden geconcludeerd dat onoplosbare vezels mest- en strooiselkwaliteit kunnen verbeteren, op voorwaarde dat dit samenvalt met een grove structuur van het voer. De effecten van het veranderen van de viscositeit of het toevoegen van een kleimineraal of laxeermiddel was niet in lijn met resultaten van eerdere studies. Op basis van de resultaten werd geconcludeerd dat het meten van A_w geen toegevoegde waarde heeft voor ons begrip van vocht in strooisel en mest, daar alle gemeten waarden dicht bij 1.0 (maximale waarde) lagen. Daarom is het meten van het totale vocht en vrij watergehalte van mest meer beschrijvend en waardevol.

De belangrijkste conclusies van dit proefschrift zijn *i*) de relatie tussen verschillen in commensaal bacteriën in gezonde vleeskuikens met commerciële voeders en mestkwaliteit lijkt beperkt, *ii*) voerformuleringen kunnen worden aangepast met de uitkomsten van de modellen uit hoofdstuk 2 om uitgescheiden nutriënten te beïnvloeden en daardoor het vochtgehalte in de mest te verminderen, *iii*) het toevoegen van Mg aan het voer kan worden gebruikt als een model om natte mest te bestuderen die veroorzaakt wordt door verminderde waterabsorptie in de darm, *iv*) effecten van onoplosbare vezels op het vochtgehalte van mest is afhankelijk van de deeltjesgrootte en *v*) het vrij water gehalte van de mest is meer geschikt als meting om mestkwaliteit te bepalen dan A_w. De implicatie van het vrij water gehalte in de mest op strooisel kwaliteit in vleeskuikenstallen dient nader onderzocht te worden.

Dankwoord

Als meisje had ik een duidelijk idee over ‘wat ik later wilde worden’. Geen zuster of juffrouw, maar professor! Uitvinder zijn zoals Einstein, hoe fantastisch is dat?! Het hoe en waarom achterhalen, dat leek me wel wat. Daarna werd ik echter “paardenmeisje” en is die droom een tijdje vervaagd. Maar mam, ik weet dat je er wel eens over in zat, maar gelukkig is het uiteindelijk toch allemaal goed gekomen! ☺ Bijna afgestudeerd aan Wageningen kwam daar toch ineens die droombaan voorbij, onderzoeker naar veevoeding. Na veel twijfelen, want wat moesten ze daar nu met mij, met een opleiding in paardenhouderij en één afstudeeropdracht met varkensvoeding (nou ja, meer hun gedrag nav voerrestrictie) en als afstudeerrichting ADP in plaats van ANU? Toch is het toen begonnen, en mijn dank is dan ook groot aan Hink en Peter dat jullie het aangedurfd hebben met mij. Het onderzoek waar ik me dagelijks mee bezig houd ligt ook op het raakvlak gezondheid en voeding (zie ook deze thesis), en is de meerwaarde van een ADP achtergrond toch gebleken. Na een jaar bij Provimi op de onderzoeksafdeling gewerkt te hebben gaf ik aan ‘ooit’ misschien wel eens een PhD te willen doen, Peter, en later ook Sander, jullie zijn daarin mijn voorbeelden geweest. Hink, nog geen maand later belde je me, ik vergeet het nooit meer: “Evelien, wil je dit nu echt, want als het niet zo is, dan moeten we aan de noodrem trekken”. Er zijn momenten geweest waarbij ik wel eens spijt had dat ik toch niet aan de rem getrokken heb, vooral in de periode dat er door mijn reguliere werkzaamheden even geen tijd meer over was voor andere activiteiten. Maar wat ben ik nu blij toch te hebben doorgezet. Thanks to Provimi for giving me the opportunity to start this PhD project and later also Cargill for giving me the opportunity to finalize this thesis.

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kun je zelf ook snel een promotie traject starten, ik weet zeker dat je er wat moois van gaat maken. Neil, your statistical advice was truly indispensable. Statistics is an entire field of knowledge by itself, but what makes you 'one of a kind' is that you have the capability to explain it to a 'SAS dummy' like me. And believe it or not, I even liked running the hundreds of models for the paper in Chapter 2, because I got so excited with the results. ☺ So "**Dankie**" for your valuable contributions.

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Tot slot, ieder is gezegend met zijn eigen talenten en we hebben de opdracht gekregen deze naar vol vermogen te benutten. Met Uw hulp.

"*A dream doesn't become reality through magic; it takes sweat, determination, and hard work*" − Colin Powell

Evelien

About the author

CURRICULUM VITAE

Evelien Hangoor was born on February 8, 1984 in Terneuzen, the Netherlands. After graduating from secondary school (HAVO, profile Nature and Health) at Zeldenrust-Steelantcollege in Terneuzen in 2001, she started her Bsc in animal husbandry at the University of Applied Sciences Larenstein in Deventer, the Netherlands. Her major specialization was equine husbandry and she did her major thesis at Massey University, Palmerston North, New Zealand, investigating genetic disorders in New Zealand miniature horses. After her graduation in 2005, she started her Msc in Animal Sciences at Wageningen University in Wageningen. Her major specialization was Adaptation Physiology, and for her major thesis she investigated the effects of mixing pigs at the start of the grower period and effects of feed restriction on grower-finisher pig behavior at the Swine Research Center of Nutreco in Boxmeer, the Netherlands. In May 2007, she started working at Provimi as a research nutritionist, where she was responsible for nutritional research in broilers and grower-finisher pigs. Having a background in animal health and physiology, intestinal health has been her interest, leading to the start of a PhD on wet litter in November 2008. The results of her study are described in the current thesis. Her employer changed to Cargill after the merger of Provimi with Cargill in 2011. After graduation, she will continue her activities as a poultry scientist for Cargill Animal Nutrition at the innovation center in Velddriel, the Netherlands.

CURRICULUM VITAE – NL

Evelien Hangoor werd geboren op 8 februari 1984 te Terneuzen. Na haar afstuderen (HAVO, profiel Natuur en Gezondheid) aan het Zeldenrust-Steelantcollege te Terneuzen in 2001, begon ze haar studie Dier- en Veehouderij aan de Hogeschool Larenstein te Deventer. Haar specialisatie was paardenhouderij en voor haar afstudeeropdracht is ze naar Massey University Palmerston North, Nieuw-Zeeland gegaan voor het onderzoeken van genetische aandoeningen in het Nieuw-Zeelandse minituurpaarden ras. Na haar afstuderen in 2005 begon ze met haar opleiding Dierwetenschappen aan Wageningen University te Wageningen. Haar hoofdspecialisatie was Adaptatiefysiologie, en voor haar afstudeeropdracht onderzocht zij de effecten van het mengen van varkens aan het begin van de mestperiode en de effecten van beperkt voeren van mestvarkens op hun gedrag bij het Swine Research Center van Nutreco te Boxmeer. In mei 2007 is ze begonnen met werken als research nutritionist bij Provimi, waar ze verantwoordelijk was voor voedingsonderzoek met betrekking tot vleeskuikens en mestvarkens. Het hebben van een achtergrond in diergezondheid en de fysiologie van dieren heeft haar interesse voor darmgezondheid gevormd, wat uiteindelijk leidde tot de start van een promotieonderzoek naar ‘natte mest problematiek’ in november 2008. De resultaten van dat onderzoek zijn beschreven in dit proefschrift. Haar werkgever veranderde in Cargill na de fusie van Provimi met Cargill in 2011. Na het afronden van haar promotieonderzoek zal Evelien haar activiteiten als pluimveeonderzoeker voor Cargill Animal Nutrition bij het proefbedrijf te Velddriel voortzetten.

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TRAINING AND SUPERVISION PLAN

Basic package (3 ECTS)

WIAS Introduction course	2009
Course on philosophy of science and/or ethics	2012

Scientific exposure (14.3 ECTS)

International conferences

23rd World Poultry Congress, Brisbane, Australia, July 1-4	2008
1st Beneficial Microbes Conference, Amsterdam, The Netherlands, May 29-30	2008
17th European Symposium on Poultry Nutrition, Edinburgh, Scotland, August 23-27	2009
2nd Beneficial Microbes Conference, Noordwijkerhout, The Netherlands, March 15-17	2010
13th WPSA European Poultry Conference, Tours, France, August 23-27	2010
Poultry Science Association Annual meeting, Denver, USA, July 12-15	2010
International conference on Responsible use of Antibiotics in Animals, Egmond aan Zee, The Netherlands, November 14-16	2011
3rd Beneficial Microbes Conference, Noordwijkerhout, The Netherlands, March 26-28	2012
24th World Poultry Congress, Bahia, Brazil, August 5-9	2012

Seminars and workshops

Themamiddag "Hennen op leeftijd", Schothorst Feed Research, The Netherlands	2009
35th Animal Nutrition Research Forum, Lelystad, The Netherlands	2010
Symposium "Innate immunity and infection", WUR - HMI, Wageningen, The Netherlands	2013

Presentations

The effect of early life feed restriction on performance and mortality of male broilers, WPC, Brisbane, Australia, July 1-4, oral	2008
Evaluating poultry excreta nutrient content by near-infrared reflectance spectroscopy, ESPN, Edinburgh, Scotland, August 23-27, poster	2009
Litter score as a predictor of excreta dry matter in broilers, ANF, Lelystad, The Netherlands, April 16, oral	2010

Correlations between excreta dry matter and nutrients in broiler by principal component analysis, EPC, Tours, France, August 23-27, poster	2010
Effect of feed composition on ileal microbiota composition in broilers, WPC, Bahia, Brazil, August 5-9, poster	2012

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Provimi Potential Managers Training (Warsaw, May 22-24)	2011
Provimi Potential Managers Training (London, October 16-18)	2011
Provimi Potential Managers Training (Barcelona, January 15-17)	2012

Research skills training (6 ECTS)

Preparing own PhD research proposal	2008/09

Didactic skills training (4 ECTS)

MSc major thesis ANU, Irene van de Linde	2009/10
MSc major thesis ANU, Corné Rademaker	2011/12

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